

Pre-crisis mouse cells show strain-specific covariation in the amount of 54-kilodalton phosphoprotein and in susceptibility to transformation by simian virus 40

(somatic variation/establishment/tumor antigen/oncogenic susceptibility/oncogenesis)

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ABSTRACT We have used several inbred mouse strains to examine the role of the 54-kilodalton (kDa) cellular phosphoprotein in transformation by the papovavirus simian virus 40. We have measured the endogenous 54-kDa phosphoprotein in cells obtained from these inbred mouse strains. To study the effect of passage, cell cultures were measured for amount of the 54-kDa phosphoprotein at the 2nd and 12th passages. In the absence of any transforming agent, the amount of endogenous 54-kDa phosphoprotein in early pre-crisis mouse cells varied in a strain-specific way. Transformation frequency varied coordinately with endogenous 54-kDa expression. Mouse strains whose cells produced a high level of endogenous 54-kDa phosphoprotein on passage did not further increase its expression after simian virus 40 transformation.

A phosphoprotein of ≈ 54 kilodaltons (kDa) is found in many different malignant cells, including cells transformed by simian virus 40 (SV40), fibrosarcomas induced by methylcholanthrene, spontaneous teratocarcinomas, Abelson murine leukemia virus- and adenovirus-transformed cells, and a variety of other transformed or tumor-derived cell lines of murine, human, and other origin (1-5). Only small amounts of host 54-kDa protein have been detected in normal mouse tissues and cell cultures (2, 6) and in embryos of different gestation periods. These small amounts are stage-specific in embryos, suggesting a role for this protein in normal mouse development, as well as in the oncogenic phenotype (7).

The gene encoding the 54-kDa phosphoprotein is one of several cellular genes whose expression may be necessary for the development of neoplasia. For example, several mouse genes influence leukemogenesis. In the high-leukemic AKR strain, two unlinked genes, *AKv-1* and *AKv-2*, regulate the expression of endogenous ecotropic viruses (8-10). Genes for Fv-1 and the H-2 complex regulate both virus production and host immune response in F₁ crosses made between AKR and low-leukemic strains of mice (11, 12). Avian leukosis virus has recently been shown to activate a cellular oncogene when it transforms fibroblasts, and most lymphomas induced by avian leukosis virus arise by provirus integration adjacent to a cellular oncogene (13). It has been reported that, in SV40-transformed cells, the 54-kDa protein is stabilized by interaction with the SV40 large tumor (T) antigen. As a result, large amounts of the 54-kDa protein are found in SV40-transformed cells (14).

Little is known about the roles of either host or viral gene products in the maintenance of the transformed state in any of these systems. We have tested the hypothesis that a cellular gene(s) regulating expression of the 54-kDa protein may be ca-

pable of influencing the effectiveness of SV40 transformation through the stabilizing interaction of the 54-kDa protein with the SV40 T antigen.

MATERIALS AND METHODS

Mice. Mice used in this study were provided by Frank Lilly (Albert Einstein College of Medicine, Bronx, NY). F₁ and back-cross mice were generated in our own colony.

Cells. At least five mice were used for each set of conditions. At 2 months of age, each mouse was sacrificed and a piece of dermis was removed from the abdomen. The tissue was washed thrice in Dulbecco's modified Eagle's medium (DME medium), minced with 0.2 ml of trypsin in a 22-cm² Petri dish, and overlaid with fresh DME medium/10% fetal calf serum. Forty-eight hours later, most tissue fragments were removed and fresh medium was added. All adherent cells were cultured thereafter in DME medium/10% fetal calf serum supplemented with penicillin and streptomycin at 100 units/ml and 100 μ g/ml, respectively. Passage of cultures was carried out in two ways. According to the 3T3 protocol (15), cultures were trypsinized and replated at 3×10^5 per 22-cm² dish every 3 days. This maintains cultures at a subconfluent density at all times. According to the Hayflick protocol (16), cultures were trypsinized and replated at a dilution of 1:10 every 7-10 days. This results in cultures that are confluent until crisis.

SV40 Infection. The infection was done with CsCl-purified banded virus (17). The cells were infected at a multiplicity of infection of 100 plaque-forming units (pfu) per cell for 2 hr at 37°C. Plates were rocked to redistribute the virus every 20 min. The amount of viral DNA taken up by the cells immediately after infection was determined by trypsinizing them and then pelleting them onto nitrocellulose paper backed by Whatman 3 MM paper soaked with 0.5 M NaOH. The nitrocellulose sheet was neutralized by inverting it cell side down onto a piece of glass and covering it successively with Whatman 3 MM paper soaked in 1.0 M Tris·HCl, pH 7.4/1.5 M NaCl for 2 min, 2 min, and 4 min. The nitrocellulose sheet carrying denatured DNA was air-dried for 10 min, baked in a vacuum oven at 80°C, and hybridized to ³²P-labeled SV40 DNA as described (18).

SV40 Transformation. After infection, cells were washed twice with DME medium, and fed with fresh DME medium/10% fetal calf serum. The next day, the cells were trypsinized and replated at 10^5 or 10^4 per 22-cm² dish. Medium was changed to 1% fetal calf serum 24 hr later, and cells were fed twice a week thereafter until dense foci were obvious, usually by 3 wk. To score the number of dense foci, plates were fixed and stained with either Giemsa or crystal violet.

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Abbreviations: kDa, kilodalton(s); SV40, simian virus 40; pfu, plaque-forming unit(s); T antigen, large tumor antigen.

To select for anchorage-independent transformants, infected and control cells were replated at 10^5 or 10^4 per dish in DME medium/10% fetal calf serum/0.33% agarose on top of 22-cm² plates previously underlaid with the DME medium/10% fetal calf serum/0.5% agarose. Colonies >0.2-mm diameter were scored at 16 \times magnification 3 wk later.

Immunoprecipitation. Primary cells were subcultured at 90% confluence. For SV40 infection or [³⁵S]methionine labeling, primary cells were passaged when confluent according to the Hayflick protocol—that is, a constant dilution of the cells (16). In some cases, the 3T3 protocol—that is, reinoculation at 3×10^5 cells per 22-cm² dish every 3 days—was also used (15). At passage 2 or 3 and at passage 12, cultures were labeled with [³⁵S]methionine for 2 hr and then extracted and immunoprecipitated with monoclonal antibody 122 (6). The immunoprecipitates were analyzed by NaDodSO₄/polyacrylamide gel electrophoresis as described (19). Monoclonal antibody 122 is specifically directed against the cellular 54-kDa protein. It does not react directly with any SV40-encoded proteins. However, it will immunoprecipitate a tight 54-kDa protein-T antigen complex from extracts of SV40-transformed cells (2, 14). The 54-kDa protein is normally phosphorylated (1-5). In the present study, this protein is phosphorylated as determined by immunoprecipitation of ³²P_i-labeled cell extracts with monoclonal antibody 122 (data not shown).

RESULTS

Quantitation of the 54-kDa Phosphoprotein in Inbred Strains of Mice. Monoclonal antibody 122 specifically detects the 54-kDa protein in mouse cells (6). Equal counts of trichloroacetic acid-precipitable material extracted from cultures of different strains were immunoprecipitated with antibody 122 and analyzed by NaDodSO₄/polyacrylamide gel electrophoresis (Fig. 1). Immunoprecipitated proteins from C57L (lanes 1, 3, and 5) and from NFS (lanes 2, 4, and 6) cells include the 54-kDa protein. At passage 3 (lanes 1 and 2), both strains show only a small

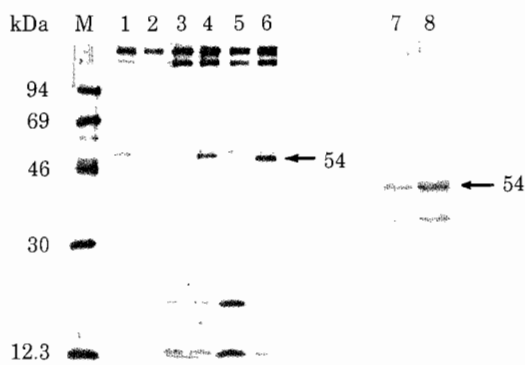


FIG. 1. Production of the 54-kDa protein by fibroblasts from C57L and NFS mouse cells. Immunoprecipitation was carried out with [³⁵S]methionine-labeled cell extracts and monoclonal antibody 122 (6) and products were analyzed by electrophoresis on a 10–20% gradient gel. Lanes 1, 3, and 5: immunoprecipitates of cell extracts from the C57L strain at passage 3, passage 12 according to the 3T3 protocol, and passage 12 according to the Hayflick protocol, respectively. No apparent change in intensity occurred at the 54-kDa protein position. Lanes 2, 4, and 6: immunoprecipitates of cell extracts from the NFS strain at passage 3, passage 12 according to the 3T3 protocol, and passage 12 according to the Hayflick protocol, respectively. There is a marked increase in intensity at the 54-kDa protein position by passage 12. Lanes 7 and 8: immunoprecipitates of cell extracts from the (C57L \times NFS) F₁ cross at passages 2 and 12. Again, a more intense band is seen at the 54-kDa position at passage 12. Equal amounts of trichloroacetic acid-precipitable radioactivity were used for all immunoprecipitations. Lane M: size markers.

amount of the 54-kDa protein. However, by passage 12, NFS cells but not C57L cells show an intense band at the 54-kDa position. Both the Hayflick (16) and the 3T3 (15) protocols resulted in large amounts of the 54-kDa protein in NFS cells by passage 12 (lanes 4 and 6).

We quantitated the passage-dependent increase of the 54-kDa protein in NFS cells by using a Gilford spectrophotometer equipped with a gel scanner. C57L cells showed no significant change from passage 3 to passage 12 by either passage protocol (Table 1). Cultures made from NFS cells showed a 22-fold increase from passage 3 to passage 12 (Table 1).

Cells from mice of the A, BALB/c, and C3H inbred strains were also examined for endogenous level of the 54-kDa protein at different passages (Fig. 2). We detected a substantial increase in the 54-kDa protein at later passage in cells of the A strain (lanes 1 and 2) and of the BALB/c strain (lanes 3 and 4). However, in the case of cell cultures from the C3H strain, 54-kDa protein levels remained low from early to late passage. Quantitatively, a 3- to 8-fold increase in the amount of 54-kDa cellular protein occurred with passage, in cells from strains A and BALB/c, while no change occurred in C3H cells (Table 1). Taken together, these results indicate that the capacity to either produce or to stabilize an increased amount of cellular 54-kDa protein on passage is intrinsic to the cells of each strain of mice.

The amounts of 54-kDa protein were also measured in some SV40-transformed cells after immunoprecipitation with monoclonal antibody 122 (Table 1). SV40 transformation abolished strain-dependent differences in the amount of 54-kDa protein. SV40-transformed C57L and C3H cells showed levels of 54-kDa protein as high as in transformed and untransformed NFS or BALB/c cells but not higher (Table 1). Apparently, detectable 54-kDa protein does not always increase in NFS and BALB/c cells on SV40 transformation. After repeated passage, untransformed cells of these strains showed as much 54-kDa protein as sister SV40 transformants.

Table 1. Quantitation of [³⁵S]methionine-labeled host 54-kDa protein in pre-crisis mouse cells

Cells	Passage	54-kDa protein immunoprecipitated*
C57L	3	0.27
C57L	12	0.30
SV40-transformed C57L [†]		5.75
NFS	3	0.30
NFS	12	6.75
SV40-transformed NFS [‡]		6.38
A [‡]	2	0.36
A [‡]	12	2.70
BALB/c	2	0.81
BALB/c	12	2.92
SV40-transformed BALB/c [†]		5.73
C3H	2	0.26
C3H	12	0.20
SV40-transformed C3H		5.58
(C57L \times NFS) F ₁	2	0.38
(C57L \times NFS) F ₁	12	4.25

Mouse dermal fibroblasts were prepared and passaged and immunoprecipitation was carried out with monoclonal antibody 122. Results presented are for cells derived from a single 2-month old mouse for each strain. This experiment was repeated with four animals of each strain with similar results.

*Total trichloroacetic acid-precipitable radioactivity ([³⁵S]methionine) was determined in each cell extract. Immunoprecipitation was done on equal numbers of counts. Amounts of the 54-kDa protein were determined from fluorograms by scanning on a Gilford 250 spectrophotometer equipped with a Gilford 2520 gel scanner (arbitrary units).

[†]After SV40 transformation, phenotypes are passage independent.

[‡]Only female mice.

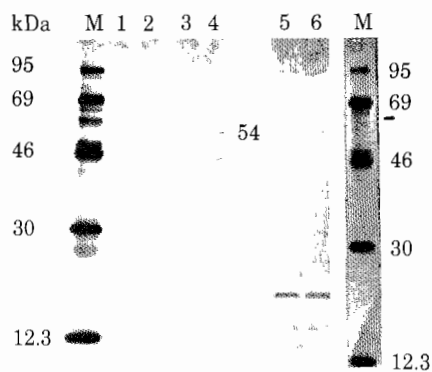


FIG. 2. Production of the 54-kDa protein by A, C3H, and BALB/c mouse cells. Immunoprecipitation was carried out with [³⁵S]methionine-labeled cell extracts and monoclonal antibody 122 (6) and products were analyzed by electrophoresis on a 10–20% gradient gel. Lanes 1 and 2: immunoprecipitates of strain A cell extracts at passages 2 and 12. Lanes 3 and 4: immunoprecipitates of BALB/c cell extracts at passages 2 and 12. A more intense 54-kDa band was detected at passage 12 in both strains. Lanes 5 and 6: immunoprecipitates of cell extracts from the C3H strain at passages 2 and 12. No increase in intensity of the 54-kDa protein band was observed for this strain at passage 12. Cells were passaged according to the Hayflick protocol. Lane M: size markers.

Dominance of Increased Expression of 54-kDa Phosphoprotein. To see whether the increase in 54-kDa protein expression with passage was dominant or recessive, F₁ animals were generated by crossing C57L and NFS strains. Cell cultures were made from 2-month-old F₁ animals and [³⁵S]methionine-labeled cell extracts were immunoprecipitated with monoclonal antibody 122. (C57L × NFS) F₁ cells showed an 11-fold more intense band of 54-kDa protein at passage 12 than at passage 2 (Fig. 1, lanes 7 and 8; Table 1). This response is similar to that of the NFS parental strain. These data are consistent with a dominant inheritance of the passage-dependent increase in the amount of 54-kDa cellular protein.

Susceptibility to SV40 Transformation of Different Inbred Strains of Mice. After transformation by a variety of viruses and chemicals, including SV40, many cells contain a high concentration of the 54-kDa protein. High levels of the 54-kDa protein are not passage dependent in transformed cells (1–5). We next transformed cells from various strains to determine whether strains showing late passage increase in production of the 54-kDa protein were more efficiently transformed by SV40. Cells were infected at early passage before any strain showed an increase in the amount of endogenous 54-kDa protein (Table 1). We infected cultures with SV40 at passage two or three, and selected transformants either as dense foci in low serum medium or as colonies in soft agarose. Because selection and cloning of transformed cells took a minimum of 8–10 passages, all transformed clones are similar in passage number to “late passage” control cells (Table 1).

Strains differed in the efficiency of transformation (Table 2). NFS and BALB/c strains were more easily transformed than other strains in both the focus forming and the anchorage assays. Cells from strain DBA/1 were anomalous. These cells were easily transformed in the density assay but were unresponsive in the anchorage assay. In all but one case, cultures made from male and female mice responded identically. In the A strain, cells from female mice were significantly less transformable than cells from male mice. This difference disappeared with further passage of the pre-crisis cells (Tables 2 and 3). With the exception of the case of female A strain cells, the strain-specific response to SV40 was identical at early and late passages (Table 3).

Table 2. Transformation frequency of different inbred strains of mice at early passage

Transformation frequency class	Mouse strain	Transformation, colonies per 10 ⁵ cells	
		Serum	Anchorage
High	AKR	4.5 ± 2.0	2.5 ± 0.7
	DBA/1	8.6 ± 1.7	0.8 ± 0.1
	DBA/2	4.1 ± 0.9	2.6 ± 1.6
	NFS	7.9 ± 1.3	3.0 ± 1.5
	BALB/c	7.9 ± 1.0	5.0 ± 1.8
	NIH	4.5 ± 0.1	7.4 ± 0.6
	A (male only)	4.8 ± 1.2	4.0 ± 0.6
Low	RF	<1.0	<1.0
	C57B10	<1.0	1.0 ± 0.1
	C57L	1.8 ± 0.5	<1.0
	C3H	2.0 ± 1.6	0.8 ± 0.1
	A (female only)	<1.0	<1.0

Skin fibroblasts made from each strain were infected with SV40 at a multiplicity of infection of 100 for 2 hr. Twenty-four hours later, the cells were plated at density of 10⁴ or 10⁵ per plate in 1% serum or 0.33% agarose. Dense foci in low serum and colonies in agarose (>0.2 mm) were scored after 3 wk. Each point is an average of at least five experiments of both male and female mice. Results represent mean ± SEM. Student's *t* test was used to determine the statistical significance of the difference between the high and the low classes of transformation frequency. For each strain, five or more animals were used for the experiment. The *t* value was always >1.40. There are eight or more degrees of freedom, which results in a probability of 90% that the difference in the high and low responders is significant.

Covariation of High Level of Endogenous 54-kDa Phosphoprotein and Efficient SV40 Transformation. Without exception, inbred strains whose cells are more highly susceptible to SV40 transformation also responded to passage by synthesizing large amounts of the 54-kDa protein (Table 4).

The response of cells from A strain female mice to SV40 varied with passage. When these cells showed very low levels of the 54-kDa protein at early passage, they also could not be transformed by SV40. However, with later passage, the level of 54-kDa protein in these cells increased and the cells also became easily transformable by SV40 (Tables 3 and 4).

Endogenous Ecotropic Virus Does Not Enhance SV40 Transformation. Mice of the AKR strain show a very high frequency of spontaneous leukemia because of the presence of endogenous retrovirus (20). Most such animals die of the disease by 1 year of age (20). Cells from AKR mice showed a transformation frequency comparable to that of other high responders (Table 2). Other high-responder strains of mice (BALB/c, NFS, NIH, etc.) do not normally express high titers of retrovirus (20). Therefore, the presence of the retrovirus probably did not have any further additive effect on SV40 transformation.

Strain-Specific Susceptibility to SV40 Is Expressed Between Virus Uptake and Abortive Transformation. We next attempted to determine the period of differential susceptibility to SV40 transformation in these strains of mice. To determine whether different transformation frequencies could be attributed to different rates or efficiencies of virus uptake, we assayed SV40 DNA in cells immediately after infection. 3T3 cells infected at 100, 30, and 10 pfu/cell showed the expected linear increase in SV40 DNA with input multiplicity, and such cells infected at 100 pfu/cell for 1, 10, 30, and 120 min showed an increase in DNA detected with increasing time of virus–cell interaction (Fig. 3). Thus, this measure of DNA uptake is sensitive both to the concentration of virus and to the time of virus exposure. Cells from NFS, C57L, and C3H strains all took up the same amount of virus, within a factor of 2 (Fig. 3), even though NFS cells can be transformed by SV40 more readily than cells from either C57L or C3H mice (Table 2). This result in-

Table 3. Transformation frequency at early and late passages

Cells	Passage	T-antigen positive at 48 hr,* %	Serum transformants		Anchorage transformants, ^{†‡} no. per 10 ⁵ cells
			Per 10 ⁵ cells [‡]	Per T-antigen positive cells, %	
C57L	3	0.24	1.8 ± 0.5	7.5	<1.0
C57L	12	0.29	1.8 ± 0.7	6.2	<1.0
NFS	3	1.05	7.9 ± 1.3	7.5	3.0 ± 1.5
NFS	12	1.60	9.6 ± 2.4	6.0	8.7 ± 2.0
A [§]	2	ND	<1.0	ND	<1.0
A	12	ND	9.0 ± 0.5	ND	8.7 ± 2.1
BALB/c	2	1.12	7.9 ± 1.0	7.1	5.0 ± 1.8
BALB/c	12	1.34	8.8 ± 0.5	6.6	7.2 ± 2.4
C3H	2	ND	2.0 ± 1.6	ND	0.8 ± 0.1
C3H	12	ND	1.3 ± 0.4	ND	0.3 ± 0.3
(C57L × NFS) F ₁	2	1.43	7.0 ± 1.3	4.9	9.1 ± 0.8
(C57L × NFS) F ₁	12	2.27	8.4 ± 0.8	3.7	20.0 ± 1.8

ND, not done.

* Immunofluorescence of T antigen was detected with monoclonal antibody to T antigen followed by antibody to fluorescein-conjugated goat anti-mouse immunoglobulin G.

[†] Results represent mean ± SEM.

[‡] Each point is an average of five separate experiments with five male and female mice. Early passage data are from Table 2.

[§] Only female mice.

indicates that the extent of virus uptake is not responsible for the differences in transformation frequency.

We next examined infected cultures for percentage of T-antigen-positive cells 48 hr after infection. By this time, strain differences were apparent (Table 3).

Transformation frequencies were proportional to incidence of T-antigen-positive cells 48 hr after infection (Table 3). Immunofluorescence of T antigen 48 hr after infection was not significantly different in early- and late-passage cultures of these strains (Table 3).

SV40 uptake by strains is approximately constant, and the number of dense foci observed per T-antigen-positive cell 48 hr after infection is also constant both for high (NFS and BALB/c)- and for low (C57L)-transformation-frequency strains (Table 3). Taken together, these data suggest that the strain-specific difference occurs in the early stage of cell-SV40 interaction. Accumulation of a large amount of 54-kDa protein-T-antigen complex (14) would be an example of such an interaction.

DISCUSSION

We have found evidence for a genetic polymorphism in both the amount of a phosphoprotein associated with transformation and the susceptibility of cells to transformation by SV40. Cell cultures from inbred mouse strains revealed inherited differences in 54-kDa protein production. One strain-specific behavior is production of higher levels of the 54-kDa protein on continued passage of dermal fibroblasts in culture. The other is the production at late passage of only trace amounts of the 54-kDa protein, similar to the amounts found in normal tissue

cells *in vivo*. Cells from strains that produce more of the 54-kDa protein with passage are more easily transformed by SV40. This distinction is apparently determined in the abortive phase of transformation, between the time of virus uptake and the time of stable DNA interaction. During the abortive phase, the SV40 T-antigen-54-kDa protein interaction may be recognized. If so, integration and later transformation events might occur at higher frequency in strains that can accumulate more of the complex soon after infection.

Comparison of the 54-kDa protein from one system with that from another is not exact at present. A cellular protein with a molecular mass of 53-54 kDa is present in large numbers of cell lines that have been either virally or chemically transformed (1-5). Similar 53-kDa proteins were detected in several human cell lines either derived from spontaneous tumors or transformed with SV40 (4, 6, 21, 22). Comparison of these 53-kDa proteins by partial proteolysis showed that though similar they were not identical in both mouse and human tissues (7, 22, 23). In one study using a cell-free translation system, 54-kDa mRNA from

Table 4. Covariation of endogenous 54-kDa protein and susceptibility to SV40 transformation

Amount of 54-kDa protein	SV40 transformation frequency	
	High	Low
High	BALB/c NFS (C57L × NFS) F ₁ A female (late passage)	None
Low	None	C57L C3H A female (early passage)

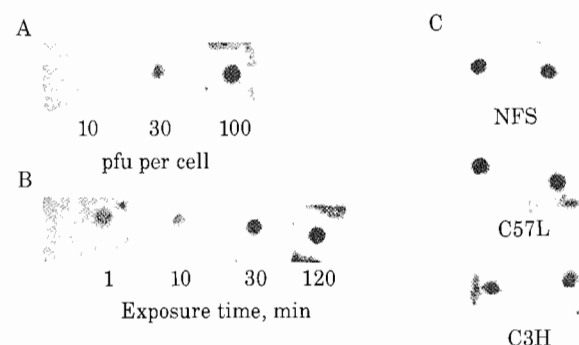


FIG. 3. Determination of SV40 uptake. After SV40 infection, early passage mouse cells were rinsed once with trypsin-containing solution, trypsinized, and pelleted onto nitrocellulose underlaid with NaOH-soaked Whatman filter paper. After neutralization and baking, the filters were hybridized to SV40 [³²P]DNA. 3T3 cells were infected with various doses of SV40 for 2 hr (A) or at a dose of 100 pfu per cell for various exposure times (B). (C) Cells from NFS, C57L, and C3H mice were infected with SV40 at 100 pfu per cell. A duplicate of each cell type is shown. All strains took up the same amount of SV40. Scintillation counting of the hybridization dots confirmed these results (data not shown).

either SV40-transformed cells or post-crisis BALB/c 3T3 cells produced roughly equivalent amounts of the 54-kDa protein. The authors of that study concluded that the 54-kDa protein made in BALB/c 3T3 cells is rapidly degraded while the protein is extremely stable in SV40-transformed BALB/c 3T3 cells. These results and others suggested post-translational regulation of expression of the 54-kDa cellular protein, perhaps requiring binding to the SV40 T antigen (2, 16, 24, 25). However, we have shown that large amounts of the 54-kDa protein are made by late-passage pre-crisis normal cells from some inbred strains of mice in the absence of SV40 T antigen. These strains were also more readily transformable by SV40. Examination of expression of the 54-kDa protein in SV40-transformed cells from passage-sensitive strains showed that transformation by SV40 did not further increase the level of 54-kDa protein. Strains that did not show elevated amounts of the 54-kDa protein on passage did yield SV40 transformants with high levels of it, as reported earlier by others (1, 2, 14, 24).

Studies of an unrelated DNA virus, Epstein-Barr virus, suggest a specific role for the viral protein-54-kDa protein complex in transformation. The major virus-encoded product regularly found in cells transformed by Epstein-Barr virus is a complex of a 48-kDa viral nuclear antigen with a cellular 53-kDa protein similar to the one detected by monoclonal antibody 122 (4).

Trace amounts of the 54-kDa protein are present in embryos of mouse, rat, and hamster. Peptide analysis has shown these proteins to be closely related to each other (6). It is not clear what role the 54-kDa cellular protein may have in embryonic development. Earlier work on transformation of rat embryo fibroblasts at different gestation periods showed that day-15 embryonic cells had a higher transformation frequency than those of day-12 or -18 embryonic cells (26). It should be of interest to see whether the level of 54-kDa protein is also highest in day-15 rat embryonic cells.

We have shown that the differences in SV40 transformation frequency observed in cells obtained from several inbred strains of mice are not due to differences in uptake of SV40. The differences were, however, manifest 48 hr after infection. Involvement of the 54-kDa cellular protein in regulation of cell growth has been reported for both embryonic and nonembryonic cells (6, 27). We therefore hypothesize that one task of the 54-kDa protein is that of acting immediately after SV40 infection, perhaps by interaction with abortive SV40 T antigen. Alternatively, both the increase in expression of the 54-kDa protein and the increase in SV40 transformation frequency may result from the expression of another dominant host gene, perhaps one involved in the establishment of cell lines after crisis. High-responder strains convert to post-crisis "3T3" lines quite easily (28, 29).

We have shown that expression of the 54-kDa protein is under host genetic control. It is important to study the host gene(s) that regulates production and stabilization of the 54-kDa protein because these genes are likely to play a role in determining the probability of an oncogenic episode in the life of an individual animal.

Transformation of established serum-transformed post-crisis NIH 3T3 cells by DNA from primary or established human tumor cells has revealed a small number of oncogenes (30-33). NFS is an inbred strain of NIH mice. During establishment of the NIH 3T3 line, the precursor cells underwent changes that most likely included enhanced expression of the 54-kDa protein (Fig. 1, lane 6). Thus, the current assay for oncogenes from various tumors is unlikely to detect any sequences except those capable of functioning in the presence of high levels of the 54-kDa protein. It would be interesting to transfect these onco-

genes into pre-crisis cells, especially those with lower levels of the 54-kDa protein, to see whether any of them are still able to cause transformation under more normal conditions.

It would also be interesting to map the genes that encode and regulate the expression of the 54-kDa protein. This could be done with the help of strains that have (e.g., NFS) and those that do not have (e.g., C57L) the capacity to express increased amounts of the 54-kDa protein.

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