Failure of Simian Virus 40 Small t Antigen To Disorganize Actin Cables in Nonpermissive Cell Lines

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Mouse C3H 10T1/2 cell lines expressing the simian virus 40 (SV40) small t antigen were obtained by cotransfection of pSV2neo and plasmids which encode small t. Cell lines derived from two plasmids which encode small t in the absence of stable deletion fragments of the large T antigen were morphologically normal and grew to slightly higher saturation densities in low serum than control cell lines. Unexpectedly, the clones had highly organized actin cables, as did parental 10T1/2 cells infected with wild-type SV40. These observations and comparisons of rat F111 cells infected with either polyomavirus or SV40 suggest that the SV40 small t antigen does not directly affect cytoskeletal organization.

Insights into simian virus 40 (SV40) small t function have been gained primarily by comparing cellular responses to infection with wild-type (WT) virus and deletion mutants. Such studies have established a role for small t antigen in enhancing transformation (3, 4, 14, 19, 22). Our laboratory has demonstrated that WT virus-infected CV1 cells are resistant to the growth-arresting effects of the drug theophylline, while deletion mutant-infected cells are susceptible (20). Association of small t antigen with 56,000- and 32,000-molecular-weight cellular proteins (56K and 32K cellular proteins) was first observed by comparing patterns of immunoprecipitated proteins from WT virus- and deletion mutant-infected CV1 cells (26). Similarly, a role for small t in continued cell cycling was suggested on the basis of the mitogenic response of mouse embryo cells to infection with deletion mutants or WT virus (11).

Not all the data suggesting functions for small t have come from these sorts of comparative experiments, however. Evidence linking small t antigen to actin cable disruption has been obtained from microinjection of small t antigen itself or of DNA fragments encoding small t (2, 9, 19).

As an alternate approach to the study of small t function, mouse C3H 10T1/2 (18) cell lines expressing small t antigen were constructed. These cell lines express small t antigen which is functional as regards to its association with the 56K and 32K proteins and which is present at levels equivalent to that seen in SV40-infected 10T1/2 cells, yet the actin cable network in these cell lines is prominent and highly organized. This observation prompted us to examine actin cables in 10T1/2 cells after infection with SV40. No disruption of actin cables was observed, and a similar result was obtained with SV40-infected rat F111 cells, although F111 cells were susceptible to polyomavirus-induced actin cable disorganization, as has been previously reported (21).

MATERIALS AND METHODS

Cells and viruses. Mouse C3H 10T1/2 cells were maintained in basal Eagle medium containing 10% fetal bovine serum (FBS). Rat F111 cells were maintained in Dulbecco modified Eagle medium (DME) containing 10% FBS. Polyomavirus and SV40 were both used as crude lysates for infection.

Plasmids. Plasmids pdl-1137 and pdl-1138 (16) were obtained from J. Pipas (University of Pittsburgh). Both plasmids contain the entire SV40 genome cloned into pBR322 at the *Bam*HI site; pdl-1137 bears a frameshift deletion at nucleotides (nt) 4454 to 4422 such that a termination codon is encountered 32 nt downstream of the deletion, and pdl-1138 bears a frameshift deletion at nt 4340 to 4310 with a termination codon at nt 4263. Plasmid pw2-t (4) was obtained from G. di Mayorca (New Jersey Medical School). In this plasmid, the early region of SV40 (nt 346 to 2533) bearing an *Hpa*I deletion (nt 3733 to 2666) is cloned into pML. Plasmid pSV2neo (23) was obtained from P. Berg (Stanford University).

Transfections. Transfections of 10T1/2 cells were done by a modification (24) of the calcium phosphate coprecipitation technique (10) with a 15% glycerol shock 4 h posttransfection. Two days after transfection, cells were plated at low density into medium containing G418 (250 µg of G418 per ml; GIBCO Laboratories, Grand Island, N.Y.) and G418-resistant colonies were picked by cylinder cloning 16 to 19 days later. Expanded colonies were maintained in 250 µg of G418 per ml for two additional passages and were maintained thereafter in the absence of drug.

Immunoprecipitation. Cells were labeled (overnight, except when indicated) with [³⁵S]methionine in medium containing 0.5% FBS and methionine at 1/18th the normal concentration. They were lysed in Trizma-buffered saline (pH 8) containing 0.5% Nonidet P-40, and a soluble protein fraction was obtained after centrifugation. To immunoprecipitate small t antigen, we added an equal volume of 2× RIPA buffer followed by a hamster antitumor serum or a mouse monoclonal antibody. Immune complexes were isolated with *Staphylococcus aureus*, Cowan strain (12). To detect the complex of small t antigen and the 56K and 32K cellular proteins, RIPA buffer was not used, and immunoprecipitation was performed with a hamster antitumor serum which had been selected for its ability to recognize the complex. For reaction with *N*-ethylmaleimide (NEM), immunoprecipitated proteins were eluted from *S. aureus* and reacted with 65 nM NEM for 1 h at 0°C, followed by the addition of 125 mM 2-mercaptoethanol and 12.5% glycerol (6).

Immunofluorescence. To visualize actin cables, cells grown on glass cover slips were fixed in 10% Formalin in

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phosphate-buffered saline and then stained with fluorescein isothiocyanate (FITC)-phalloidin (5 μ g/ml in phosphate-buffered saline; Sigma Chemical Co., St. Louis, Mo.) (25). To examine large T antigen expression, cells were fixed in absolute methanol, incubated with pAb419 (a monoclonal antibody which recognizes both SV40 T antigens) or anti-PyT54 (hamster antiserum against all three polyomavirus T antigens, generously supplied by D. Simmons), and then washed and incubated with FITC-conjugated goat anti-mouse or FITC-conjugated goat anti-hamster immunoglobulin (Organon Teknika, Malvern, Pa.). For double-immunofluorescence staining, cells were fixed in 10% Formalin, permeabilized with 0.1% Nonidet P-40 in phosphate-buffered saline, and then reacted sequentially with FITC-phalloidin, pAb419, and rhodamine-conjugated goat anti-mouse immunoglobulin (Organon Teknika), washing extensively with phosphate-buffered saline after each incubation.

Transformation. Subconfluent monolayers of rat F111 cells were infected with virus and then trypsinized and seeded at 10^5 cells per 60-mm dish in 0.3% agar (Difco Laboratories, Detroit, Mich.) over a bottom layer of 0.5% agar (in DME-10% FBS). Dishes were incubated at 37°C, and colonies larger than 0.1 mm in diameter were counted 5 to 6 weeks later.

RESULTS

Establishment of 10T1/2 cell lines expressing small t antigen.

10T1/2 cells were cotransfected with pSV2neo and an eight-fold excess of a small-t-encoding plasmid (pdl-1137, pdl-1138, or pw2-t) and subcultured into medium containing G418, and the G418-resistant clones were picked and expanded. After an overnight pulse with [35 S]methionine, clones were screened for expression of small t antigen by immunoprecipitation and sodium dodecyl sulfate (SDS)-gel electrophoresis. Control clones were obtained by cotransfection with pBR322 and pSV2neo. Similar numbers of G418-resistant clones were obtained from all cotransfections.

One-fourth to one-half of all clones tested expressed detectable levels of small t antigen. Those clones which expressed the highest levels of small t antigen had levels of small t comparable to those seen in SV40-infected 10T1/2 cells at 2 days postinfection (Fig. 1A). Clones derived from transfection with pdl-1137 also expressed high levels of a 14,000-molecular-weight protein, presumably a stable large T fragment which is expressed owing to the frameshift deletion of pdl-1137 (Fig. 1B, lane 2). When cells were lysed under conditions in which most of the large T remains associated with the nucleus, this fragment was found entirely in the cytoplasmic portion of the lysate (data not shown).

Owing to the position of the frameshift deletion in pdl-1138, small-t-expressing clones derived from transfection with this plasmid could also potentially express an 18,000-molecular-weight truncated large T protein of the same size as small t antigen. To detect this putative large T fragment, we reacted immunoprecipitates of cell extracts from pdl-1138-derived cell lines with NEM before SDS-gel electrophoresis. NEM alkylates sulfhydryl groups, which are abundant in cysteine-rich small t antigen, and such alkylation retards the migration of small t in gel electrophoresis (6). The truncated large T protein is cysteine poor, and its mobility would not be expected to be altered by reaction with NEM. Electrophoresis of immunoprecipitated proteins from pdl-1138-derived clones which had been pulsed for 75 min revealed an 18,000-molecular-weight band apparently com-

FIG. 1. Immunoprecipitation of small-t-expressing 10T1/2 clones. Cells were pulsed overnight with [35 S]methionine. Cell lysates were immunoprecipitated with the monoclonal antibody pAb419, and the immunoprecipitated proteins were analyzed by SDS-polyacrylamide gel electrophoresis. Equivalent counts per minute from each lysate were used for immunoprecipitation in panel A but not in panel B. (A) Lanes: 1, control clone derived from transfection with pBR322; 2, clone 72 derived from transfection with pdl-1138; 3, SV40-infected 10T1/2 cells (MOI, 100); 4, clone 9 derived from transfection with pw2-t. (B) Lanes: 1, WT SV40-infected CV1 cells; 2, clone AA4 derived from transfection with pdl-1137; 3, clone 50 derived from transfection with pdl-1137del. 14K, 14,000-molecular-weight fragment.

posed of two polypeptides, only one of which migrated more slowly after reaction with NEM (Fig. 2, compare lanes 3 and 4). The 18,000-molecular-weight fragment which remained after NEM treatment was not visible when cells were pulsed overnight before immunoprecipitation and NEM treatment, suggesting that this band represents an unstable fragment.

Small-t-expressing clones derived from transfection with pw2-t showed no evidence of a truncated large T fragment even after a brief pulse. A fragment encoded by this plasmid would have an expected size of 40,000 molecular weight, and the pw2-t-derived small-t-expressing clones showed no unique bands in this region of the gel when compared with control clones (Fig. 2, lanes 7 and 8).

Characteristics of cell lines derived from transfection with pdl-1137. All clones which expressed both small t antigen and the stable, abundant 14,000-molecular-weight large T fragment exhibited a distinctive, retracted morphology when compared with both control clones and the parental 10T1/2 cell line (Fig. 3). Two days after being plated at low density in 10% FBS, 90% of cells from a control clone were well spread, whereas 100% of the examined cells from a pdl-1137-derived clone appeared spindly and retracted.

Growth rates and saturation densities of the pdl-1137 clones were similar to those of control clones. When the

FIG. 2. Detection of unstable large T fragments in small-t-expressing 10T1/2 clones. Cells were pulsed for 75 min with [35 S]methionine in methionine-free medium. Cell lysates were immunoprecipitated with pAb419, which recognizes both SV40 T antigens. For extracts shown in lanes 1 to 6, each lysate was divided in half before immunoprecipitation. Immunoprecipitates were reacted (lanes 2, 4, and 6) or not reacted (lanes 1, 3, and 5) with NEM before analysis by SDS-polyacrylamide gel electrophoresis. Lanes: 1 and 2, control clone derived from transfection with pBR322; 3 and 4, clone 73 derived from transfection with pdl-1138; 5 and 6, SV40-infected CV1 cells. After alkylation by NEM, small t antigen migrates to the position indicated by t'. Immunoprecipitates in lane 7 (control clone derived from transfection with pBR322) and lane 8 (clone 9 derived from transfection with pw2-t) were not reacted with NEM.

cells were maintained at confluence with periodic feeding, the continued viability of the monolayers varied with the serum lot. In those sera in which viability was maintained, continual sloughing of cellular material from the monolayer was observed, although the monolayer as a whole remained confluent and showed no signs of detaching from the dish. This unusual behavior at confluence was not seen in any of the control cell lines.

To determine whether the characteristic morphology and behavior at confluence of the pdl-1137 clones was due to the presence of the abundant large T fragment, we constructed a plasmid which encoded this truncated fragment in the absence of intact small t antigen. This plasmid, pdl-1137del, was constructed by digesting pdl-1137 with *Bst*XI, which cuts toward the C-terminal end of small t (nt 4759), and then digesting the linearized plasmid for a brief period with *Bal* 31 nuclease. Religated plasmids were screened by restriction enzyme analysis, and a plasmid which contained a 200-base-pair deletion around the *Bst*XI site but which retained the splice acceptor site was obtained. 10T1/2 clones expressing high amounts of the 14,000-molecular-weight large T fragment were isolated after transfection with this plasmid (Fig. 1B, lane 3). These clones showed a characteristic retraction (Fig. 3) and exhibited cell sloughing when held at confluence, although at a reduced level when compared with that of

FIG. 3. Morphology of 10T1/2 clones expressing a 14,000-molecular-weight fragment of large T antigen. Phase-contrast photographs (magnification, $\times 250$) of cells, 2 days after plating at low density in DME-10% FBS. (A) Control clone derived from transfection with pBR322; (B) Clone AA2 derived from transfection with pdl-1137; (C) clone 50 derived from transfection with pdl-1137del.

clones expressing both small t and an equivalent level of the truncated fragment.

Characteristics of cell lines derived from transfection with pdl-1138 or pw2-t. All clones derived from transfection with either pdl-1138 or pw2-t were morphologically indistinguishable from control clones and did not accumulate cell debris in the medium when held at confluence. Growth curves of the two clones expressing the highest levels of small t antigen, pw2-t 9 and 1138 72, were compared with growth curves of control clones and the parental 10T1/2 cells. Cells were plated at low density in 60-mm dishes and then maintained in either 5 or 0.5% serum with twice-weekly feeding. Cells were trypsinized and counted every 4 days.

During the log phase of growth in either 5 or 0.5% serum, small-t-expressing and control clones showed very similar patterns. In 5% serum, wide variations in saturation densities were seen between clones, with no apparent pattern distinguishing t-antigen-positive and -negative clones. However, when cells were maintained in medium containing

TABLE 1. Growth of small-t-expressing 10T1/2 clones in 0.5% serum^a

Clone	Cell count (10 ⁵)			
	Day 4	Day 8	Day 12	Day 16
pw2-t 9	2.9	8.2	11.0	15.2
1138 72	3.4	7.5	8.3	9.2
322 4	3.4	5.0	5.3	5.4
322 10	2.4	5.0	5.3	5.4
10T1/2	2.1	4.2	4.8	4.9

^a Cells were plated in 60-mm dishes at 5×10^4 cells per dish in DME containing 10% FBS. The next morning, the medium was changed to DME containing 0.5% FBS, and cells were fed with this same medium twice weekly. At each time point, cells in duplicate dishes were trypsinized and counted in a Coulter Counter.

0.5% serum for up to 16 days, the small-t-antigen-expressing clones grew to a somewhat higher density than control clones (Table 1). Clones expressing lower levels of small t did not show these higher saturation densities in 0.5% serum.

Our laboratory has previously shown that two cellular proteins, 56K and 32K, are immunoprecipitated along with small t antigen when certain hamster antisera are used to immunoprecipitate extracts of WT virus-infected CV1 cells (26). The same complex of small t antigen and the 56K and 32K proteins was also immunoprecipitated from extracts of small-t-expressing 10T1/2 cells (Fig. 4). These cellular proteins were not seen in immunoprecipitates of extracts of control clones. In addition, mixing experiments showed that small t antigen from 10T1/2 cell lines was able to associate with the 56K and 32K cellular proteins from monkey CV1 cells. Thus, as regards its association with cellular proteins, the small t antigen being expressed by the 10T1/2 clones is functional.

To determine whether expression of small t antigen in 10T1/2 cells affected the state of actin cable organization, cells were grown on cover slips and then fixed and stained with FITC-phalloidin. Cells from both of the clones expressing the highest levels of small t antigen exhibited distinct and extensive actin cable networks, identical to the pattern seen in cells of control clones (Fig. 5). In contrast, a 10T1/2 clone derived from transformation with polyomavirus middle T antigen exhibited a total absence of organized stress fibers when stained with phalloidin (Fig. 5).

Actin cables are not disrupted immediately after SV40 infection of 10T1/2 cells and rat F111 cells. Because levels of small t antigen expression equivalent to those seen in SV40-infected 10T1/2 cells were not associated with actin cable disruption, the effect of SV40 infection on actin organization in 10T1/2 cells was examined. Twenty-four hours after infection of subconfluent 10T1/2 cells with WT SV40 (100 to 200 PFU per cell), cells were fixed and stained for actin cables or for large T antigen expression. While nearly half of the cells stained positive for large T, virtually none of the cells showed actin cable disruption, exhibiting a pattern indistinguishable from that of mock-infected cells (Fig. 6).

Previous studies have documented a significant loss in organized actin cables immediately after infection of rat F111 cells with polyomavirus (21). Since rat F111 cells are nonpermissive for both SV40 and polyomavirus, morphological changes and actin cable patterns in F111 cells infected with either polyomavirus or SV40 could be compared. F111 cells were infected with polyomavirus or with SV40 which had been concentrated by sedimentation such that its titer (3×10^9) was fivefold greater than that of our polyomavirus

stock. Immunofluorescent staining for large T antigen (polyomavirus or SV40) indicated that the majority of cells was infected. Polyomavirus-infected cells appeared retracted within 24 h after infection, while SV40-infected cells retained an appearance identical to that of mock-infected cells. Infection with polyomavirus resulted in actin cable disruption in almost every cell examined, as indicated by the fuzzy, indistinct pattern of staining by phalloidin (Fig. 7B), whereas SV40-infected and mock-infected F111 cells had identical actin-staining patterns at 24 h postinfection. Double-labeled immunofluorescence staining of SV40-infected F111 cells with phalloidin and a monoclonal antibody against SV40 large T antigen revealed that even cells with the most intense nuclear fluorescence exhibited a pronounced actin cable network (Fig. 7C and D). Thus, although infection with polyomavirus results in an immediate and dramatic effect on actin cables in F111 cells, SV40 infection does not appear to perturb this cytoskeletal component.

FIG. 4. Association of the 56K and 32K cellular proteins with small t antigen in small-t-expressing 10T1/2 clones. Cells were pulsed overnight with [³⁵S]methionine. Cell lysates were immunoprecipitated with a hamster antiserum which recognizes the complex of small t antigen and the 56K and 32K cellular proteins. Immunoprecipitates were analyzed by SDS-polyacrylamide gel electrophoresis. Lanes: 1, clone derived from transfection with pBR322; 2, clone 9 derived from transfection with pw2-t; 3, clone 72 derived from transfection with pdl-1138. Lanes 4 to 7 represent mixing experiments in which lysates from [³⁵S]methionine-labeled uninfected CV1 cells were incubated before immunoprecipitation with unlabeled lysates from a clone derived from transfection with pBR322 (lane 4), clone 9 derived from transfection with pw2-t (lane 5), clone 72 derived from transfection with pdl-1138 (lane 6), or SV40-infected CV1 cells (lane 7).

FIG. 5. Actin filaments in small-t-expressing 10T1/2 clones. Cells were grown on glass cover slips, fixed in 10% Formalin, and stained with FITC-phalloidin. Magnification, $\times 250$. (A) Clone derived from transfection with pBR322; (B) clone 9 derived from transfection with pw2-t; (C) clone 72 derived from transfection with pdl-1138; (D) clone derived from transformation with polyomavirus middle T antigen.

To compare the transforming capabilities of these same virus stocks, F111 cells were infected with polyomavirus or SV40 at equivalent multiplicities of infection (MOIs) and then plated immediately into soft agar. Six weeks later, the resulting anchorage-independent colonies were counted and categorized by size (Table 2). Polyomavirus infection resulted in a two- to fourfold-higher total number of colonies than did SV40 infection, and a greater proportion of polyomavirus-induced anchorage-independent colonies fell into the higher size categories; however, the two viruses did not differ dramatically in their capacities to transform rat F111 cells.

DISCUSSION

Cell lines expressing small t antigen were obtained by cotransfection of pSV2neo and one of three small-t-encoding plasmids into 10T1/2 cells. The frequency with which G418-resistant clones appeared, the frequency with which these drug-resistant clones also expressed small t antigen, and the range of levels of small t expression were similar for all three plasmids. The presence and stability of truncated large T antigens did vary with the plasmid used for transfection, however. For cell lines derived from transfection with pdl-1137, large amounts of a stable 14,000-molecular-weight fragment were expressed along with the small t antigen. A stable fragment encoded by a similar plasmid has been reported previously (17). In cell lines derived from transfection with pdl-1138, which bears a small frameshift deletion just 100 base pairs downstream from the deletion in pdl-1137, an unstable 18,000-molecular-weight large T fragment was

coexpressed with small t. In cell lines obtained after transfection with pw2-t, no large T fragments were detected. The deleted region of pw2-t includes the large T translation termination codon, and no other termination codon is present in the remainder of the message. Thus, the mRNA which encodes this putative 40,000-molecular-weight fragment may not be translatable.

Cell lines which expressed high levels of the 14,000-molecular-weight truncated large T antigen exhibited altered morphology and abnormal behavior at confluence, particularly in some lots of serum. The fragment did not appear to affect plating efficiencies or growth rates. At confluence, pronounced sloughing of cells from an underlying intact monolayer was observed. This was more pronounced in cells which coexpressed the 14,000-molecular-weight fragment and small t antigen than in cells which expressed equivalent levels of the fragment only, suggesting that small t antigen

TABLE 2. Colony formation in soft agar of rat F111 cells infected with SV40 or polyomavirus^a

Expt	Virus	Colonies/ 4×10^5 cells		
		0.1–0.5 mm	0.5–1.0 mm	1.0–1.5 mm
1	Polyomavirus	187	53	2
	SV40	153	12	0
2	Polyomavirus	352	21	4
	SV40	82	0	0

^a Cells were infected with SV40 or polyomavirus at an MOI of 100 and were plated immediately into soft agar at 10^5 cells per dish (four dishes each). Colonies were scored 5 to 6 weeks later. Experiments 1 and 2 used different serum lots.

FIG. 6. Actin filaments in SV40-infected 10T1/2 cells. Cells were plated on glass cover slips at 10^5 cells per 35-mm dish and infected with SV40 at an MOI of 100 to 200. Twenty-four hours later, cells were either fixed in methanol and stained for large T antigen with pAb419 and FITC-conjugated goat anti-mouse immunoglobulin or fixed in 10% Formalin and stained with FITC-phalloidin. Magnification, $\times 250$. (A) Mock-infected 10T1/2 cells stained for actin filaments. (B) SV40-infected 10T1/2 cells stained for large T antigen. (C) SV40-infected 10T1/2 cells stained for actin filaments.

contributes to this behavior. It was not possible to determine whether cells died and detached from dishes, followed by replacement cell divisions, or whether cell division continued at confluence with consequent shedding of excess cells into the medium.

Cell lines obtained with pdl-1138 and pw2-t were morphologically normal and showed no deviation from normal growth behavior except for the capacity to undergo one additional round of division when grown to confluence in 0.5% serum. Cell lines which express the polyomavirus small t antigen have been described by others (5, 15). These grew to higher cell densities than control clones, even in

medium containing 10% serum. Our clones which expressed the SV40 small t antigen and two SV40 small-t-expressing clones derived from BALB/c 3T3 cells with a retroviral vector (1) did not show higher saturation densities in 10% serum.

The most surprising characteristic of these 10T1/2 cell lines was their retention of normal actin cable organization. The small t antigen expressed by these clones was functional with respect to its ability to associate with the 56K and 32K cellular proteins. A clone of small-t-expressing BALB/c 3T3 cells, kindly provided to us by D. Livingston, was also examined for actin cable organization. This clone, B2.8.2, showed enhanced susceptibility to transformation by suboptimal levels of SV40 large T antigen (1). Similar levels of small t antigen were detected in this clone and in 10T1/2 clone pw2-t 9, and the actin cable pattern of clone B2.8.2 was identical to that of the parental 3T3 cells (unpublished observations).

Our findings regarding actin cable structure in small-t-expressing clones prompted us to examine the acute effects of SV40 infection on actin cable organization. Mouse 10T1/2 cells were infected with SV40 at a multiplicity sufficient to infect the majority of the cells, yet no perturbations in the actin cable network were detected. Rat F111 cells were also used so that SV40 and polyomavirus infection could be compared in nonpermissively infected cells. At similar MOIs, only polyomavirus induced pronounced loss of cables. Previous studies have shown that the polyomavirus mutant 808A, which expresses large T and small t antigens only, does not disrupt actin cables after infection of NIH 3T3 cells (13). F111 cells transformed by a middle T cDNA plasmid lack actin cables (13), and we obtained a similar result with middle-T-transfected 10T1/2 cells, implying a role for polyomavirus middle T in actin cable disruption.

Although polyomavirus disrupted actin cables in rat F111 cells while SV40 did not, the two viruses did not show dramatic differences in their abilities to transform this same cell line. A previous study which compared the abilities of polyomavirus and SV40 to transform F111 cells showed a similar result (7). However, the ability of polyomavirus to cause an immediate cytoskeletal reorganization may contribute to its somewhat higher efficiency of transformation and the more vigorous growth of the transformed colonies in agar.

Only one previous study has examined actin cables immediately after infection with SV40. In this study, which used mouse embryo cells, 80% of the infected cells lacked distinct actin cables 48 h after infection with WT SV40 (11). We also examined SV40-infected primary mouse embryo cells, using double-labeled immunofluorescence so that the same cell could be scored for both nuclear T antigen and actin cable organization. Using this technique, we also found an association between SV40 infection and loss of cable organization. A minority of the cells showed intense nuclear fluorescence, and 70 to 75% of these lacked distinct actin cables. This association was not apparent when nuclear fluorescence was scored as weak, and 25 to 45% of mouse embryo cells with no nuclear staining also showed lack of organized cables. Thus, disruption of actin cables in acute SV40 infection may be cell-type specific, may require very high levels of early protein expression, and may occur in cells which are particularly susceptible to cable disorganization.

One approach which has suggested a correlation between SV40 small t expression and actin cable disorganization has been the study of transformants selected after infection with either WT SV40 or small t antigen deletion mutants (8). Such

FIG. 7. Actin filaments in SV40- and polyomavirus-infected rat F111 cells. Cells were plated on glass cover slips at 10^5 cells per 35-mm dish and infected with SV40 at an MOI of 5,000 or with polyomavirus at an MOI of 1,000. Twenty-four hours later, cells were fixed in 10% Formalin and stained with FITC-phalloidin (A and B) or fixed in Formalin, permeabilized with Nonidet P-40, and stained sequentially with FITC-phalloidin, pAb419, and rhodamine-conjugated goat anti-mouse immunoglobulin G (C and D). Magnification, $\times 250$. (A) Mock-infected F111 cells; (B) polyomavirus-infected F111 cells; (C and D) SV40-infected F111 cells, same field stained for both large T antigen (C) and filamentous actin (D).

an approach cannot distinguish secondary cellular alterations which occur as a result of transformation from immediate effects of the viral proteins on the cells. In our laboratory, anchorage-independent colonies isolated after WT virus infection of rat F111 cells were examined for actin cable patterns. Most, but not all, of the transformants did lack organized actin cables.

Another approach was microinjection of either small t protein or DNA fragments which could encode this protein. In these studies, the levels of small t antigen present in the cells may greatly exceed levels which can be achieved by infection and which are sufficient for cellular transformation. Overall, these considerations and the observations reported in this paper suggest that small t does not play a direct role in disorganization of actin cable structures and that caution should be exercised in attributing biological effects of small t antigen to alteration of the cellular cytoskeleton.

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