

# African Green Monkey Fibroblast Actin Morphology during SV40 Infection

Gerhard Brandner and Myung-Sam Cho

Institut für Virologie, Universität Freiburg

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Monkey skin fibroblasts were infected with simian virus 40. Cells that exhibited the viral tumor antigen were found to retain the normal morphology of actin filaments up to six days after infection. However when cells were transformed in terms of focus formation they had lost the normal actin morphology.

## Introduction

In cell culture, non-transformed fibroblasts exhibit well-organized bundles of microfilaments, a major portion of which is actin. Cell transformation by simian virus 40 (SV40)<sup>1</sup> or Rous sarcoma virus<sup>2</sup> results in a significant decrease in the expression of the actin filaments. In the case of chick fibroblasts transformed by a temperature sensitive (ts) Rous sarcoma mutant virus, such changes of the actin filaments have been shown to be controlled by viral transforming genes: a shift from restrictive to normal temperature was followed by an actin pattern rearrangement within one hour<sup>2</sup>.

In the case of SV40, transformation experiments with ts mutant viruses<sup>3–5</sup> and other data<sup>6</sup> have provided evidence that gene A, the early viral genome region, is responsible for the viral transforming ability. Microinjection experiments with SV40 cRNA<sup>7</sup>, the temperature sensitivity of expression and properties of tumor (T) antigen in cell transformed by SV40 ts A mutant virus<sup>8,9</sup> as well as SV40 mRNA *in vitro* translation data<sup>10</sup> provided evidence that gene A codes for the T-antigen. Thus, SV40 infection of susceptible cells begins with the expression of gene A and the subsequent appearance of T-antigen in the cell nucleus. After a few weeks and some cycles of cell division the transformation of a fraction of the cells can occur. Such SV40-transformed fibroblasts have lost their actin filaments, in addition to acquiring a number of typical transformation features.

We have now investigated whether the appearance of T-antigen in monkey skin fibroblasts, which

are non-lytically infected by SV40, is paralleled by the loss of the actin filaments or whether this loss is observed only in conjunction with transformation.

## Materials and Methods

### Virus

Two SV40 strains were used in parallel: strain 777 and strain ELO. Strain ELO was isolated from a human infant<sup>11</sup>. The viruses were propagated on primary African green monkey (AGM) kidney cells. For transformation studies, partially inactivated virus strain ELO was used: A 2 ml aliquot (in a Petri dish) of a suspension of this virus ( $10^8$  TCID<sub>50</sub> ml<sup>-1</sup>) was irradiated with UV light ( $2 \text{ mW cm}^{-2}$  for 1 min).

### Cell culture

The experiments were performed with uncloned AGM skin fibroblasts. The cells were propagated from skin biopsies in Eagle's MEM containing 5% fetal calf serum and were used after 4 to 6 passages.

### Transformation of AGM skin fibroblasts with UV-irradiated SV40

Semiconfluent skin fibroblast cultures (area  $40 \text{ cm}^2$ ) were infected with 10 ml of irradiated virus suspension containing the equivalent of  $10^7$  TCID<sub>50</sub> ml<sup>-1</sup> of native virus. The cultures were trypsinized and propagated after a 1:3 dilution on the first and on the ninth day after infection. When foci of transformed cells appeared (10 to 50 foci per culture after 2–4 weeks) the cultures were trypsinized and reseeded on cover slips. Uninfected control cultures were processed similarly. After reaching semi-confluence the cells were rinsed with saline and fixed in acetone ( $4^\circ\text{C}$ , 10 min).

Requests for reprints should be sent to Dr. G. Brandner, Institut für Virologie der Universität Freiburg, Postfach 820, D-7800 Freiburg.



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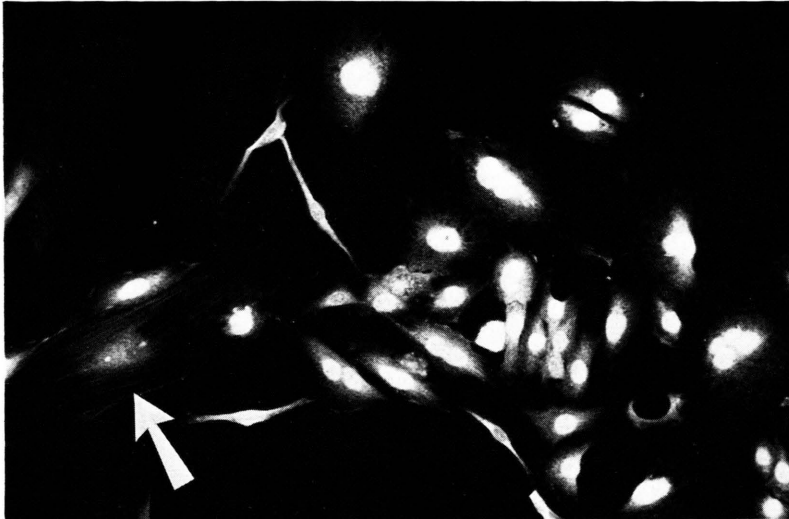


Fig. 1. Absence of actin filaments in SV40(ELO) transformed monkey skin fibroblasts. Foci of transformed cells were passaged five times without further cloning. The cultures were stained for T-antigen with monkey antiserum and for actin with human antiserum in a double staining test as described. The fluorescence both of T-antigen and actin was exposed on the same film. The picture shows one T-antigen negative normal fibroblast exhibiting cytoplasmic actin filaments (arrow) and several T-antigen positive transformed cells (bright nuclei) lacking actin filament morphology.



Fig. 2. Presence both of T-antigen and actin filaments in a monkey skin fibroblast 6 days after infection with SV40(777). The cell culture was stained for T-antigen with monkey antiserum and for actin with rabbit antiserum in a double staining test as described. The photographs (a) and (b) show the same cell in the light of (a), the actin filament and (b), the T-antigen immunofluorescence.

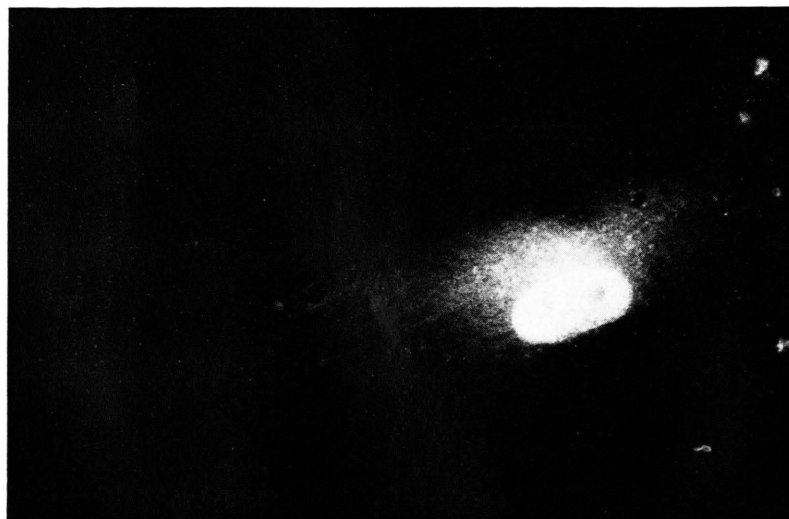


Fig. 2 a.

Fig. 2 b.

*Non-lytic infection of monkey fibroblasts*

The cells were grown on cover slips ( $24 \times 24$  mm<sup>2</sup>) to semiconfluency and infected with  $0.5$  to  $1 \times 10^8$  TCID<sub>50</sub> ml<sup>-1</sup> of SV40 strain 777 or ELO. Control cultures were mock infected. After a 3 h adsorption period fresh medium containing 5% fetal calf serum was added. The cells were incubated for 2, 4 and 6 days and fixed as above.

*SV40 T- and capsid-antigen and actin detection*

The indirect immunofluorescence technique was used. Antiserum against T-antigen was from a monkey immunized with homogenates from SV40-transformed AGM kidney cells or from SV40 tumor-bearing Syrian hamsters. Anti SV40 capsid serum was from a SV40-infected AGM; anti T-antigen antibodies had been eliminated from this serum by absorption with acetone dry powder of SV40-induced hamster tumors. For the second step we used anti monkey and hamster globulins labeled with rhodamin. Anti actin sera were from selected human patients with chronic active hepatitis<sup>12</sup> (a gift from Dr. H. Berthold, Institute of Virology and from Dr. G. Haag, Medizinische Universitätsklinik). In some tests we used rabbit anti chicken gizzard smooth muscle actin serum (kindly donated by Dr. U. Gröschel-Stuart, Zoologisches Institut, Technische Hochschule, Darmstadt). This antibody exhibits a brilliant fluorescence with smooth muscle cells and a moderate fluorescence with subcultured skin fibroblasts whereas primary skin fibroblasts are not stained with this antibody (U. Gröschel-Stuart, personal communication).

The anti globulins (from goat and swine) for the second step were labeled with fluoresceine isothiocyanate. For simultaneous detection of SV40 T-antigen and actin in fibroblast cultures on the same cover slip the respective first and second antisera were combined. Each of the two reactions were carried out for 1 h at 37 °C. The fluorescence was observed and photographed with a Zeiss photomicroscope equipped with an epifluorescence condenser type III RS.

**Results***SV40 T-antigen and actin filaments in transformed AGM skin fibroblasts*

Skin cell cultures exhibiting foci of SV40-transformed cells were seeded on cover slips and the mixed cell population was examined for the presence of both T-antigen and actin by means of two colour immunofluorescence. Uninfected controls were monitored in parallel. We could detect the

Table I. Expression of T-, capsid-antigen, and actin filaments in SV40 infected, transformed, and normal monkey skin fibroblasts.

Experiment	T-antigen positive cells	Capsid-antigen positive cells	Fraction of well-spread cells	Well-spread cells exhibiting actin filaments [%]
	[%]	[%]	[%]	
cells uninfected	0	0	70	95–98
SV40 infected cell 6 days p.i.	5–20	5–20	70	95–98
SV40 transformed cells	98	0	70	1–2

typical actin filaments in most of the T-antigen negative fibroblasts that were well-spread (Table I). In contrast, cells which had T-antigen positive nuclei exhibited a more round shape and no or very weak and blurred actin filaments (Fig. 1). In a separate test, no cell was found positive for SV40 capsid-antigen.

*SV40 T-antigen and actin filaments in non-lytically infected fibroblasts*

AGM skin fibroblasts infected with SV40 strain 777 and ELO exhibited T-antigen fluorescence in 2% to 4% of the cells 2 days and in 5% to 20% of the cells 6 days after infection. Some cells had vacuoles. However, significant cell culture destruction was not observed. Well-spread fibroblasts were examined for the expression of the actin filament and the simultaneous presence of T-antigen in the same cell until 6 days after infection. We observed in the majority of those cells the typical actin morphology (Table I, Fig. 2). This means that infection with SV40 and expression of the nuclear T-antigen had no effect on the actin filament morphology up to 6 days after infection.

**Discussion**

We have demonstrated that infection of semi-permissive monkey skin fibroblasts with partially inactivated SV40 resulted in transformation of a small cell fraction. These cells were transformed according to focus formation and presence of T-antigen and were found to have lost the actin filaments that are a feature of un-transformed fibroblasts.

This alteration of the actin morphology is characteristic of virus transformation of some cell lines studied so far and was also observed in SV40 transformed mouse 3T3 cells<sup>1</sup>. In addition to those monkey skin cells that have already undergone transformation in terms of focus formation, we have investigated whether in monkey skin cells infected with SV40 the actin filament morphology was altered concomitantly with or subsequent to the expression of T-antigen. However, we have found that practically all well-spread cells exhibited a normal type of actin filaments whether or not they possessed the nuclear T-antigen.

Only a small fraction of infected T-antigen positive cells is "destined" after several cell cycles finally to become transformed<sup>13</sup>. Because that minority of cells cannot be identified among the T-antigen positive cells, we cannot rule out that those few cells exhibit a loss of actin filament as soon as they exhibit SV40 T-antigen.

In summary, if the loss of the actin filaments is defined to be a sufficient criterion of cell transformation, then the inability of SV40 T-antigen to cause a loss of the actin filaments early after infection could mean that the majority of SV40 T-antigen positive monkey skin fibroblasts exhibited no signs of an "abortive transformation" during this period.

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