Expression of Virus Specific Morphological Cell Transformation Induced in Enucleated Cells

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Chick embryo fibroblasts infected by a temperature sensitive mutant of Rous sarcoma virus were enucleated with Cytochalasin B. The cytoplasts were still able to transform morphologically when shifted from nonpermissive to permissive temperature, and to revert to normal morphology when shifted from permissive to nonpermissive temperature. This indicates that the viral gene product responsible for transformation acts primarily on cytoplasmic or membrane components and not on the nucleus.

The continuous action of a viral gene product is necessary for the maintenance of cell transformation induced by Rous sarcoma virus (RSV). This has been shown with chicken cells infected with mutants of RSV temperature sensitive for cell transformation. Such cells, when cultivated at nonpermissive temperature, appear normal by morphology and by other criteria. Transformation can reversibly be induced by a shift to the permissive temperature in an average time of about 6 hours. (For review see ref. 1).

The intracellular site of action of the viral gene product responsible for transformation is not known. Since membrane alterations as well as an increased division rate have been observed in transformed cells, its action could be on the membrane/cytoplasm, the nucleus, or both. In the present paper we demonstrate that nuclear components are not required for the function of the transformation-inducing viral gene product as judged by the change of cell morphology.

The cells used in all experiments were of the TaSp7 strain. This strain was derived from chick embryo fibroblasts (from the SPAFAS flock) infected with the Ta-RSV mutant 2, kindly provided by Dr. J. P. Bader, Bethesda, Maryland. Colonies were produced at 35 °C in semisolid medium containing methylcellulose, isolated and grown up in calf serum + 0.5% chicken serum, on coverslips (25 mm in diameter) from Thermanox (Eurolab, Wiesbaden). To correlate individual cytoplasts in stained preparation to those observed in video-recordings, the coverslips were coated with the negative image of electronmicroscopic finder grids (consisting of lettered hexagonal fields), produced by platinum shadowing (unpublished). Morphological changes of the cytoplasts were evaluated by redrawing their outline on the T.V. screen at different phases of the experiment, after replay of the time lapse recordings. This made it possible to follow the fate of individual cytoplasts. A decrease in size combined with an increased refractility or darkening of the cytoplast body was used as criterion for transformation and the converse parameters for normalization. Due to the poor optical quality of the video-recordings and to the contrast reduction caused by the platinum coat, those cytoplasts which could not be clearly classified as responding or not responding to a temperature change were scored negative. The percentages given are therefore only approximate and represent minimum estimates. Enucleation was essentially performed as described by others 3, using 10 μg/ml Cytochalasin B (Serva, Heidelberg) in maintenance medium. The cells growing on coverslips were centrifuged face down on stainless steel grids for 15 min in a Sorvall centrifuge at 12 100 × g and at about 35 °C.

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** Fig. 1 see Plate on page 768 a.
Two types of experiments were performed: TaSp7 cells of normal or of transformed phenotype were enucleated and tested for their ability to respond to temperature shifts. In the first experiment, cells of the normal phenotype were used: a coverslip culture with TaSp7 cells grown at 35 °C for 19 hours was shifted to 41 °C 5 hours previous to enucleation with Cytochalasin B. After the enucleation procedure, the coverslip was washed 3 times with 5 ml of medium (41 °C) and placed in a petri dish containing maintenance medium. After incubation at 41 °C for 1 1/2 hours most of the "cells" had recovered from the effect of the drug and appeared to be successfully enucleated. At this time, time lapse recordings were started to be made from an area with 2 complete and 3 incomplete hexagons containing 142 identifiable cytoplasts, most of them shown in Fig. 2 a. The culture was then shifted to 35 °C. Within 1 1/2 hours 40% of the cytoplasts underwent a morphological transformation (Fig. 2 b). Thereafter, the cytoplasts were shifted back to 41 °C. After another 1 1/2 hours 90% of the cytoplasts which had previously "transformed" had again acquired a normal morphology (Fig. 2 c). After a third shift to 35 °C for 45 min, still 35% of the cytoplasts underwent a morphological transformation. Pictures in Fig. 2 a–c represent a sequence showing the same areas with enucleated cells. The photographs were taken from the screen of a T.V. set after replay from a videorecorder. Two hexagons, indexed 0 with 1 dot (lower left) and 0 with 3 dots (upper left), are shown in addition to two hexagon halves at the right side. The pictures shown in the insets in a–c were taken 10 min before the low magnification-shots, also demonstrating the high motility of the cytoplasts. The detailed morphological changes as studied by time lapse recording following the fate of individual cells were very similar to those observed with nucleated cells. The completeness of the enucleation as suggested by the appearance of the living cytoplasts was corroborated by staining. Only 3 nucleated cells were present in the area observed (Fig. 2 d). Another area of the grid, containing 132 cytoplasts, was evaluated at the times indicated in Fig. 2 and showed similar percentages of morphologically altered cells.

In the second experiment, TaSp7 cells grown at 35 °C for 20 hours were enucleated, washed and allowed to recover as above but at 35 °C. Most of the cytoplasts now showed a transformed morphology. An area containing 72 identifiable cytoplasts was then time lapse-recorded and followed during 1 hour after shift up to 41 °C, 1 hour after shift down to 35 °C and 1 further hour back to 41 °C. A normalization of 90% of the cytoplasts was observed after the first shift, a transformation of 90% after the second, and a normalization of 45% after the third shift. The coverslip used in the experiment was subsequently stained: only 2 nucleated cells were found in the area recorded previously.

As controls, uninfected and wild type RSV-transformed chick embryo fibroblasts were grown and enucleated as the TaSp7 cells of normal and of transformed phenotype, respectively. No temperature dependent morphological changes correlating with transformation or normalization could be observed in the cytoplasts from these control cultures. That the TaSp7 cytoplasts were capable of undergoing major morphological rearrangements is not surprising in view of the fact that cytoplasts in other cell systems have been found to be viable for 1–3 days and to be capable of movement and protein but not DNA or RNA synthesis (for review see refs 3, 4).

With the approach taken in our experiments, 4 possible results could have been expected: a requirement of the nucleus for a) transformation and normalization, b) transformation only, c) normalization only and d) neither transformation nor normalization. The demonstration that cytoplasts of TaSp7 cells undergo a temperature dependent transformation and normalization rules out the first 3 possibilities suggesting that the primary target for the transforming gene product does not reside in the nucleus but in the cytoplasm or in the membrane. Since the morphology of a cell appears to be largely determined by the organization of its cytoskeleton, this could be the possible target, as has also been proposed recently by Edelman and Yahara. However, it remains to be shown that alterations of the microfilament-microtubuli system indeed take place in enucleated TaSp7 cells shifted from one temperature to the other.

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* Fig. 2 see Plate on page 768 b.
Fig. 1. TaSp7 cells (a) grown at 41 °C for 1 hour after shift from 35 °C, (b) grown at 35 °C for 1 hour after shift from 41 °C, (c) shifted back to 41 °C for another 11/2 hours. Pictures a—c represent a sequence showing the same cells. (d) TaSp7 cells grown at 41 °C and stained (May Grünwald-Giemsa). The rectangles indicate the area enlarged in the insets.

Bar represents 100 μm; in inset, 50 μm.
Fig. 2. TaSp7 cells enucleated at 41 °C. (a) At 41 °C, 1½ hours after recovery from Cytochalasin B. (b) 1½ hours after shift to 35 °C. (c) 1½ hours after shift back to 41 °C. (d) Cytoplasts from the same field at the end of the experiment, after a further shift to 35 °C for 45 min, stained as in Fig. 1. The arrows indicate the only remaining nucleated cells in the area shown. The magnifications are as in Fig. 1.