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FUSED CELLS ARE SUITED FOR MICROINJECTION

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SUMMARY

Volumes transferred per cell by microinjection are enhanced by a factor of 10^4 to 10^5 when multinucleated HeLa cells fused by polyethylene glycol 1000 are used as recipients instead of single HeLa cells. Fused HeLa cells are viable and support simian virus 40 gene expression upon microinjection of the viral DNA as do the mononucleated parental cells.

INTRODUCTION

Microinjection of mammalian tissue culture cells is a very sensitive and reproducible technique for the assay of viral and non viral macromolecules (1-6). The biological activity of the injected material (DNA, RNA, proteins) can be tested either directly in the recipient cells by means of immunofluorescence, autoradiography, complementation studies etc., or in transformed cell lines derived from injected cells (1-7; A. Graessmann, unpublished observations). If biological material has to be isolated from the recipient cells, several thousands of cells have to be injected since only 10^{-11} to 10^{-10} ml can be transferred per mammalian tissue culture cell (2).

To circumvent this difficulty we asked whether fused cells can be used for microinjection. PEG induces fusion of mononucleated cells to huge multinucleated cells (8). HeLa cells fused by PEG 1000 were used in this preliminary study to investigate the feasible injection volume of giant cells, their viability and their ability to support SV40 gene expression.

Abbreviations: PEG, polyethylene glycol; SV40, simian virus 40; DME, Dulbecco's modified Eagle's medium; FBS, fetal bovine serum; DNA I, superhelical DNA form I; cRNA, complementary RNA; T-antigen, tumor antigen; PBS, phosphate buffered saline; SDS, sodium dodecyl sulfate.

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MATERIALS AND METHODS

Cell fusion. Optimal fusion conditions have to be tested for each individual cell line. Variables are (a) the choice of the fusion agent, e.g. PEG 1000, PEG 6000, inactivated Sendai virus, (b) concentration and (c) time of action of the fusion agent, (d) density of cells before fusion and (e) mode of washing of cells after fusion. In our experiments, HeLa cells were grown to confluence in DME supplemented with 5% FCS. For fusion, the medium was removed and 1 to 2 ml PEG (49% PEG 1000 (Sigma) in DME, w/v) were added per 60 mm dish. After 1 to 2 min the PEG solution was aspirated and cells were washed twice with DME containing 15% DMSO (v/v) and three times with DME. Cells were then incubated at standard conditions and washed again 2 to 5 h later with DME supplemented with 5% FCS.

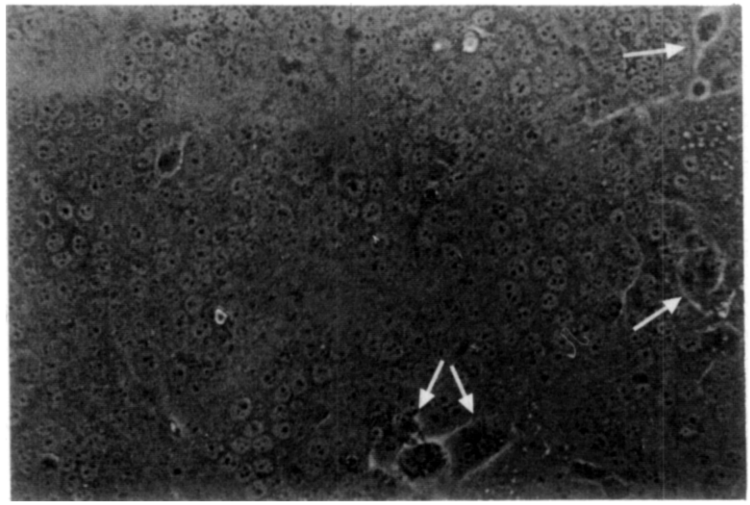
SV40 DNA and SV40 cRNA. SV40 DNA was extracted from CV1 monkey cells 45 h after infection with SV40 strain 776 by the method of Hirt (9) and further purified to SV40 DNA I by centrifugation to equilibrium in CsCl density gradients containing ethidium bromide (200µg/ml) as already described (2,3). [^{3}H]-Uridine labeled early SV40 specific cRNA was obtained by in vitro transcription of SV40 DNA I with E.coli DNA dependent RNA polymerase (2,10).

Microinjection. Microinjection was performed by our microinjection technique under a phase contrast microscope by means of glass microcapillaries (1,11).

Immunofluorescence. SV40 T-antigen was visualized by direct immunofluorescent staining of fixed cells with Tetramethylrhodamine coupled hamster IgG directed against the antigen (12).

RESULTS AND DISCUSSION

HeLa cells fused to multinucleated cells as described in Materials and Methods were estimated to contain between 500 and several thousand nuclei (Picture 1). The volume feasible to inject into these cells was determined by measuring the cpm recovered in the lysate of a 60 mm dish after microinjection of tritiated dATP into the cytoplasm of fused cells. 10µCi of [8-^{3}H]-dATP were freeze-dried, dissolved in 20µl PBS, and the solution used for microinjection of 10 randomly chosen giant cells. Directly after inoculation, cells were washed three times with PBS and lysed with 0.5 ml 1% SDS in PBS (w/v). The lysate, combined with two additional washings with 0.5 ml 1% SDS and dissolved in 10 ml scintillation cocktail (Toluol-PPO-POPOP/Triton-X100), contained 4.4×10^3 cpm. When the injection solution was released into the culture medium by placing the micropipette directly above the cell layer, only 110 cpm were found in the lysate. Since 10^{-3} ml of the injection solution yielded 2.6×10^5 cpm upon addition to the lysate of an uninjected HeLa culture, the volume transferred per fused cell was calculated to be about 2×10^{-6} ml.

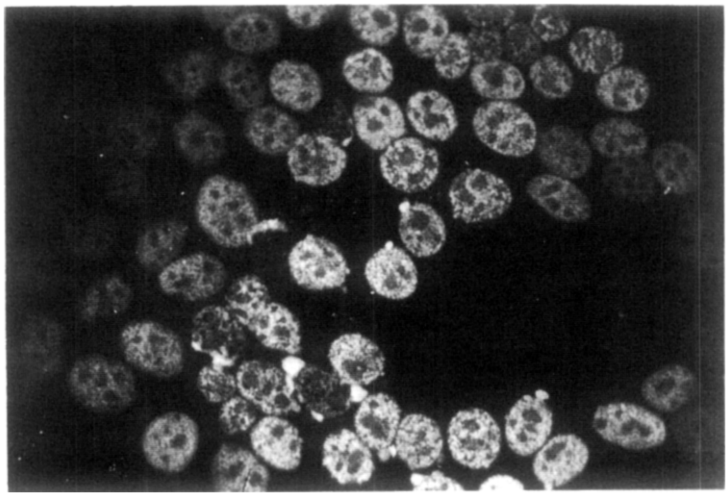


Picture 1. Partial view of a fused HeLa cell with several hundred nuclei. Arrows show unfused cells. Phasecontrast, 200x magnification.

Injection volumes of the same order of magnitude were found in independent experiments where ^{3}H-labeled SV40 cRNA was reisolated from the cytoplasm of microinjected fused HeLa cells. In short, cRNA (1 mg/ml; 1-2x10^{8} cpm/mg RNA) was injected into giant HeLa cells 4 h after fusion. Cells were washed with PBS, lysed with Nonidet-40 (0.5%, v/v, in 150 mM NaCl, 1 mM EDTA, 10 mM Tris -HCl, pH 7.5) and the nuclei pelleted at 500xg for 10 min. The supernatant was adjusted to 0.2% SDS, extracted with phenol, and the RNA precipitated on 22 mm filter discs with trichloroacetic acid. Between 5x10^{2} to 5x10^{3} cpm were recovered from single injected giant cells, corresponding to an injection volume of 5x10^{-6} to 5x10^{-5} ml.

To test their viability, fused HeLa cells were microinjected with SV40 DNA I. 15 h later, cells were fixed and stained for T-antigen. On the average, 7 out of 10 injected giant cells stained positive, indicating that this fraction of fused cells had survived microinjection and the following incubation period. Almost all nuclei within the surviving injected cells exhibited a strong T-antigen specific fluorescence (Picture 2). Occasionally a few T-antigen positive mononucleated cells were detected, these were possibly regenerated from fused cells.

Cell fusion is a technique frequently employed in virology (13). As demonstrated, this technique can be adapted for microin-



Picture 2. T-antigen positive multinucleated HeLa cell (partial view), fixed and stained 15 h after SV40 DNA injection. The nuclei are located in different planes, therefore some are out of focus.

jection: fused cells are viable after microinjection for sufficient periods of time, they support expression of transferred DNA as does the parental cell line, and they tolerate large injection volumes. It should now be possible to follow the fate of transferred material biochemically after injection of a few individual fused cells. We have shown recently that SV40 (and polyoma virus) cRNA is translated into biologically active T-antigens upon microinjection into mammalian tissue culture cells (2,14). These experiments did not prove whether the injected RNA has to be modified (spliced, capped, polyadenylated) before translation. This issue can now be settled. Moreover, cytochalasin B enucleated cells can be fused with PEG 1000 and microinjected (data not shown). This system may allow the discrimination between nuclear and cytoplasmic modification steps.

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