Biophys. Struct. Mechanism 1, 329—332 (1975)

Short Communication

Changes of Cell Surface Configuration and Regulation of Cell Proliferation*

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Received August 1, 1975 / Accepted October 27, 1975

Abstract. The electric surface charge configuration of 3T3 and SV40-3T3 cells was characterized by determining the product of electrophoretic mobility of the cells times the viscosity of suspension medium. This quantity could be shown to change with temperature and/or treatment with calf serum or trypsin in close correlation with the effects of these agents on characteristics of cell proliferation. The present results, taken together with those of earlier studies on cell-electrophoresis and characterization of lipid constituents of the cells, support the hypothesis of a lateral phase separation in the plasmamembrane as triggering process in stimulation of proliferation of resting normal cells.

Key words: Normal and Transformed Fibroblasts — Cell-Electrophoresis — Temperature Transition — Cholesterol-Content.

The plasmamembrane of cells appears to be intrinsically involved in processes of triggering or blocking of cell division (Noonan and Burger, 1974). Configurational changes of the plasmamembrane in relation to regulation of cell division have hitherto mainly been probed by plant agglutinins such as concanavalin A (Ben-Bassat *et al.*, 1971; Burger, 1971). However, there has arisen evidence that the plant lectins not only probe, but simultaneously alter the configuration of the cell membrane (Noonan and Burger, 1970; Block *et al.*, 1974; Barnett *et al.*, 1974). We have used therefore the method of micro-cell-electrophoresis to characterize the surface charge configuration as an indicator for configurational changes of the plasmamembrane of 3T3, 3T6, SV40-3T3, PY-3T3 cells (Adam and Adam, 1973, 1975). Using this method, the suspended cells are subjected to electric fields of about 5 V cm^{-1}, which may be presumed not to affect the physical state of the plasmamembrane. The product of electrophoretic mobility u_E of the cells and the viscosity η of the suspension medium is determined as a characteristic of the cellular surface charge configuration.

In the previous work, we have shown that 3T3-cells suspended by treatment with EDTA exhibit a thermal transition of the electrical surface charge configuration at about 20° C. After mild trypsin-treatment of the EDTA-released 3T3-cells, the surface charge configuration was found to be independent of temperature between 5° C and 40° C and of a magnitude, which corresponds to the

* Paper presented at: Biomembran Symposium der Sektion Zell- und Membranforschung der Deutschen Gesellschaft für Biophysik, Freiburg 17./18. 4. 1975.

low temperature configuration of the untreated cells. This result was interpreted as a shift upon trypsin-treatment of the temperature transition of surface charge configuration to temperatures above 40° C. In the case of SV40-3T3 cells, trypsin-treatment could not affect the surface charge configuration at temperatures above 25° C. Protease treatment has been shown to stimulate proliferation in resting 3T3-cells (Burger, 1971). Our results might thus indicate a major configurational change in the plasmamembrane being functionally related to stimulation of cell division. An effect of stimulation of 3T3-cells by pronase-treatment, however, could not be obtained in the experiments of Glynn *et al.* (1973).

In order to check if other stimulants of cell proliferation in 3T3-cells yield a similar effect as trypsin-treatment, we have now extended our micro-electrophoresis studies to an investigation of EDTA-released 3T3 cells, treated with calf-serum. Stimulation of proliferation of confluent 3T3-cells by serum-treatment appears to be undisputed and could be verified for the cell preparations used in the present study, which upon treatment of quiescent cells at a density of about $3 \cdot 10^4$ cells/cm^2 with fresh medium containing 33% or 50% calf serum within about 24 hrs double their cell number, whereas controls remain quiescent.

Since our earlier studies exhibited an effect of calcium concentration in the suspension medium on electrophoretic mobility of SV40-3T3 cells, we have included this ion in the suspension media of the present study in order to approximate physiological ionic milieu.

Cells (Swiss-3T3 and SV 101-3T3) and growth conditions are the same as described by Adam *et al.* (1975)

The experiments are done as described earlier (Adam and Adam, 1975); further experimental details are given in the legend to the figure.

As the results of the figure show, the presence of 1 mM Ca does not affect the temperature dependence of the surface charge configuration of EDTA-released 3T3 cells, *i.e.* a thermal transition at about 20° C is observed as in the earlier study.

Upon treatment with serum, an effective stimulant of proliferation in resting 3T3 cells, this transition is shifted to a region of higher temperatures, located at about physiological growth temperature (37° C). Since the cells were washed extensively before harvesting and after serum treatment (Adam and Adam, 1975), binding of serum to the cells appears very unlikely as contributing to its observed effect on $(u_E \cdot \eta)$ of 3T3-cells. Furthermore, it has been shown in experiments performed at 25° C (Adam and Adam, 1975), that treatment with serum prior to EDTA-release from the plates has the same effect on $(u_E \cdot \eta)$ as the reverse sequence of treatments employed in the present study. This indicates that EDTA-release of the cells from the plates does not interfere with the effect of serum on the cell membrane. Comparing the present results on the effect of calf serum on plasmamembrane configuration of 3T3-cells with those obtained earlier on the effect of mild trypsin-treatment, we may conclude that both agents of stimulation of cell proliferation have a very similar effect on the electric surface charge configuration. This supports the notion of a functional relation between the configurational change in the membrane, as probed by the electric surface charge, and stimulation of growth.

In the figure is shown also the effect of trypsin-treatment on the surface charge configuration of SV40-3T3 cells in the presence of Ca in the suspension medium: at

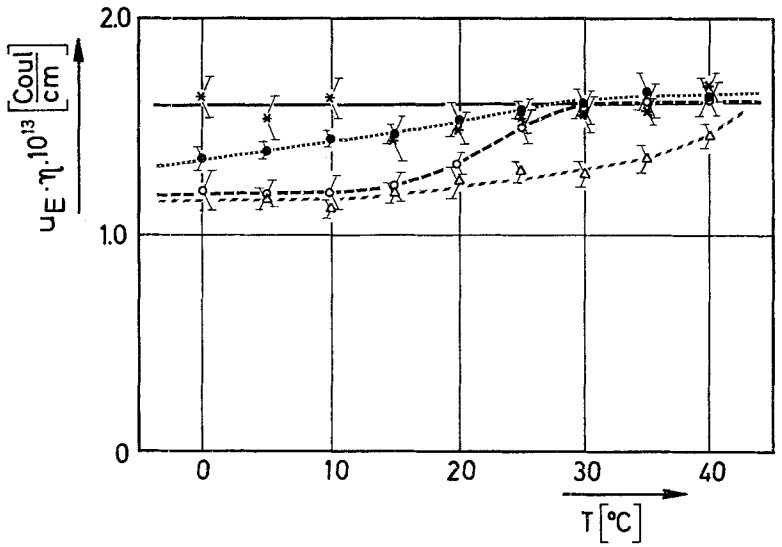

Fig. 1. Plot of product $u_E \cdot \eta$ (u_E = electrophoretic mobility, η = viscosity of suspension medium) versus temperature T. Open circles: EDTA-released 3T3-cells, open triangles: EDTA-released 3T3 cells treated with 50% calf serum in Dulbecco-Eagle medium at 37° C for 30 min, full circles: EDTA-released SV40-3T3 cells, asterisks: EDTA-released SV40-3T3 cells treated with 70 μg/ml trypsin at 37° C for 5 min. Measurements were made in 1 mM Ca^{++}/Mg-free Dulbecco's phosphate buffered saline at pH 7.2. Bars indicate standard error of mean, each point representing 3 to 6 independent experiments

temperatures above 25° C there is no effect of trypsin treatment on $(u_E \cdot \eta)$, confirming the result obtained earlier in the absence of divalent cations.

For EDTA-released SV40-3T3 cells a very broad thermal transition (width ≈ 30° C) is observed in contrast to the relatively narrow transition (width ≈ 10° C) observed for EDTA-released 3T3 cells (see Fig. 1). This finding may be correlated with a cholesterol content per cm^2 cell surface of about 50% higher in SV40-3T3 and PY-3T3 cells as compared to 3T3 cells (Adam *et al.*, 1975). As it is known from model studies using lamellar phospholipid/cholesterol mixtures, that relatively small increases of cholesterol content broaden the gel to liquid-crystaline transition drastically (Chapman, 1973), our above findings suggest that the thermal transitions of surface charge configuration are indications of thermal transitions involving the lipid-matrix of the plasmamembrane.

In conclusion our present results show:

i) Stimulants of cell division show a correlation in their effect on the temperature dependence of the cell surface charge configuration and in their known effect on proliferation of 3T3 and SV40-3T3 cells: in 3T3 cells the thermal transition is shifted to about growth temperature, whereas in SV40-3T3 cells there is no effect above 25° C.

ii) The thermal transition of surface charge configuration observed for untreated 3T3 cells is much narrower than for untreated SV40-3T3 cells, which correlates with their cholesterol contant per cm^2 cell surface. This indicates a participation of membrane lipids in the phenomena discussed.

Thus, the present results, taken together with our previous results on characterization of cellular lipids (Adam *et al.*, 1975) support the hypothesis that cell division in normal confluent cells by serum- or trypsin-treatment is triggered via a lateral phase separation in the plasmamembrane (Adam and Adam, 1975) and that the deviation in regulation of cell division of transformed cells may be attributed to an altered characteristic of lateral phase separation, as indicated by the broadening of the thermal transition in transformed cells. This altered regulation characteristic may be due to a higher cholesterol content.

Acknowledgement. The present work was supported by a grant of the Deutsche Forschungsgemeinschaft to Sonderforschungsbereich 138 „Biologische Grenzflächen und Spezifität".

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