Correction. In the article “Differences in Membrane Fluidity and Structure in Contact-Inhibited and Transformed Cells” by Ronald E. Barnett, Leo T. Furcht, and Robert E. Scott, which appeared in the May 1974 issue of the Proc. Nat. Acad. Sci. USA 71, 1992-1994, the authors request that the following changes be made. On page 1992, in the right-hand column, the first sentence of the third paragraph should read, “The organization of membrane lipids in contact-inhibited and transformed 3T3 fibroblasts was probed with a mixture of 3 spin labels, methyl-6-(4′,4′-dimethyloxazolidinyl-N-oxyl)-heptadecanoate (10%), N-(1,1-dimethyl-2-hydroxyethyl)-6-(4′,4′-dimethyloxazolidinyl-N-oxyl)-heptadecamide (30%), and 1-[2-(1,1-dimethyl-2-hydroxyethyl)imino]cyclopentyl]-1-(4′,4′-dimethyl-oxazolidinyl-N-oxyl)-undecane (60%).” On page 1993, in the left-hand column, the following sentence should be added to the end of the first paragraph: “The observed differences in the order parameter could also be due to differential uptake or metabolism of the labels by the cells.”
Differences in Membrane Fluidity and Structure in Contact-Inhibited and Transformed Cells*

(electron paramagnetic resonance/freeze fracture electron microscopy/viral transformation/cell proliferation)

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ABSTRACT Studies on contact-inhibited mouse embry fibroblast 3T3 cells and 3T3 cells transformed by oncogenic RNA and DNA viruses and by a chemical carcinogen have demonstrated differences in plasma membrane architecture. Spin-label and freeze-fracture ultrastructural studies have shown that contact-inhibited cells have ordered membrane lipids and aggregated intramembranous particles, whereas transformed cells have fluid membrane lipids and randomly distributed intramembranous particles. These findings suggest a model for how changes in the cell membrane may account for some of the characteristic differences observed between contact-inhibited and transformed cells.

Differences in the physical and biochemical characteristics of the plasma membrane of normal and transformed cells have been demonstrated. Transformed cells show greater agglutinability with concanavalin A (1, 2), increased rates of transport (3, 4), and loss of contact inhibition (5). Freeze fracture studies have also shown that intramembranous particles, which are thought to represent intrinsic membrane proteins (6), are aggregated in contact-inhibited cells and dispersed throughout the membrane in transformed cells. These observations were interpreted to suggest that differences in membrane structure may be associated with differences in membrane protein–lipid interactions and in membrane fluidity (7–9). It is plausible that many of the plasma membrane alterations observed in transformed cells are due to such changes in the fluidity and organization of membrane lipids and proteins. For example, the Na+,K+-ATPase, which has been identified with the plasma membrane sodium pump, has been shown to be affected by lateral phase separations or phase transitions in the membrane lipids (10). A similar mechanism may affect the activity of membrane enzymes that control the intracellular levels of cyclic nucleotides (11–13). In this paper we report direct experimental evidence for differences in membrane fluidity and structure of normal and transformed cells, and propose a model for the regulation of cell proliferation by modulation in the organization of the cell membrane.

METHODS AND RESULTS Contact-inhibited Balb/c and Swiss 3T3 mouse fibroblasts and simian virus 40 (SV), polyoma virus (PY), murine sarcoma virus (MSV), and methylcholanthrene (MC)-transformed Balb/c 3T3 fibroblasts (a gift of Drs. Todaro and Aaronson) were grown in Dulbecco’s minimal essential medium in 10% calf serum with penicillin (100 IU/ml) and streptomycin (10 mg/ml). 3T3 cells transformed by SV are designated SV3T3, etc. Cells were incubated at 37° in a humidified atmosphere containing 10% CO2. Cells were routinely passed by treatment with a solution of ethylenediaminetetraacetate (EDTA) (0.05 mM) and trypsin (0.025%). All cell lines used in this study were found to be free of mycoplasma contamination as determined by thin section and freeze-etching electron microscopy (14).

Cell samples were prepared for freeze fracture by glycrrination with or without prior fixation in 1% glutaraldehyde–phosphate-buffered saline, pH 7.4 for 5 min at 37°. Freeze fracture was performed in a Balzers BAE 300 freeze-etch microscope at -100°, 10−4 torr. Platinum–carbon replicas were analyzed in a Philips EM 300 electron microscope, and the percentage of fracture faces containing aggregates was determined as previously described (7).

The organization of plasma membrane lipids in contact-inhibited and transformed 3T3 fibroblasts was probed with the spin label sodium 6-(4',4'-dimethyloxazolidinyl-N-oxyl)-heptadecanoate (I) (10). A 0.3 mM solution of the label in the standard medium or in the phosphate-buffered saline, pH 7.4, was incubated with the cells for 30 min at 37° (15). Cells were then washed five times with the buffered saline, pH 7.4, and finally with label-free Dulbecco’s minimal essential medium. The cells were gently scraped from the culture flasks and centrifuged at 60 × g to form a loose pellet which was transferred to the capillary portion of a pasteur pipet, which served as the sample cell. The electron paramagnetic resonance (EPR) spectra of the cells were recorded at 33° with a Varian E-3 spectrometer. The labeling procedure had no adverse effects on cell viability, as less than 5% of the cells were trypan blue positive before and after the labeling procedure.

Order parameters, S, were calculated from the EPR spectra (16). Values of S near 1 correspond to an ordered state of membrane lipids, while values near 0 correspond to a fluid state. With the label used in this study, a ΔS = 0.03 is equivalent to a change in membrane fluidity produced by a 6–10° temperature change (10). Representative EPR spectra of transformed cells with an order parameter of 0.54 and of contact-inhibited cells with an order parameter of 0.61 are shown in Fig. 1a and b.

The data in Table 1 clearly demonstrate that the membranes of transformed cells are more fluid than the membranes of normal contact-inhibited cells. Cells which are highly con-

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tact-inhibited have an order parameter of 0.60–0.61, while
the order parameter of transformed cells is 0.52–0.55. These
differences in membrane fluidity are not due to differences in
growth rate because transformed MSV3T3 cells with an order
parameter of 0.54 have a longer generation time than contact-
inhibited Swiss 3T3 cells, with an order parameter of 0.61.
Mouse fibroblasts transformed with DNA viruses (SV3T3
and PY3T3), and RNA virus (MSV3T3), and a chemical
carcinogen (MC3T3) show similar differences in fluidity when
compared to contact-inhibited cells.

Differences in the architecture of the plasma membrane of
contact-inhibited and transformed cells have also been ob-
served (Table 1 and Fig. 2). Freeze fracture studies show that
contact-inhibited cells have aggregated intramembranous
particles and that cells transformed with DNA viruses (7),
RNA viruses, and chemical carcinogens contain randomly
distributed intramembranous particles.

FIG. 1. EPR spectra of Balb/c SV3T3 (clone A31, subclone
6) fibroblasts (a) and contact-inhibited Balb/c 3T3 (clone A31)
fibroblasts (b) labeled with spin-label I and recorded at 33°C.

DISCUSSION

Analysis of the EPR spectra of spin-labeled fatty acids in
natural membranes results in the conclusion that the labels
have two important characteristics: (1) The labels must un-
dergo rapid anisotropic motion about an axis approximately
parallel to the fatty acid chain with a correlation time of less
than $10^{-7}$ sec (10, 17–21) and (2) the labels must under-
go rapid lateral diffusion with a diffusion constant of $10^{-5}$–$10^{-4}$
cm$^2$/sec (22–25). Since rapid axial motion and rapid lateral
diffusion could not occur if the labels were bound to proteins,
these characteristics support the conclusion that the labels are
in a phospholipid-bilayer-like environment. It has been shown,
for example, that the lateral diffusion of surface antigens
throughout the cell membrane in mouse–human hetero-
karyons requires approximately forty minutes (26), while spin
labels of fatty acids can be calculated to require about one
second for complete diffusion in similar size cells.

The studies of Linden et al. suggest that the membrane
lipid bilayer is organized in a mosaic of liquid and ordered
patches (27) in the physiological temperature range. Since
membrane proteins have an ordering effect on the membrane
lipids (10, 28) and prefer an ordered environment, membrane
proteins must preferentially partition into the more ordered
patches of the mosaic.

The greater order of plasma membrane lipids observed in
this study in contact-inhibited cells reflects an increase in the
fraction of the membrane lipids in an ordered state. This
could result from an increase in the number of ordered patches
or from an increase in the size of patches in the membrane.
Modulation of the size and number of ordered patches in the

FIG. 2. Freeze fracture replica of Balb/c SV3T3 (clone A31,
subclone 6) fibroblasts (a) and contact-inhibited Balb/c 3T3
(clone A31) fibroblasts (b) × 22,000.

| TABLE 1. Differences in membrane fluidity and structure of
contact-inhibited and transformed cells |
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FF, fracture faces; IMP, intramembranous particles.
membrane may result from aggregation of certain membrane proteins or from primary changes in membrane lipids. Detailed freeze fracture studies have shown that intramembranous particles, which probably represent membrane proteins, are distributed in large aggregates in the plasma membrane of contact-inhibited 3T3 cells. Since transformed cells have more fluid lipids, it is reasonable to suggest that the ordered patches are smaller. Consistent with this suggestion is the observation that the intramembranous particles are not aggregated in transformed 3T3 cells but are randomly distributed. Further studies are necessary to establish the precise relationship between aggregation of intramembranous particles and the ordering of membrane lipids, as are studies to establish the precise distribution of the spin label in cell membranes. Nevertheless, the present data make it possible to correlate differences in the membranes of normal and transformed cells, including differences in membrane fluidity, the distribution of intramembranous particles, the relative levels of cellular cyclic nucleotides (11-13), and membrane transport (3, 4). From these observations one can suggest a model for the regulation of certain cell membrane activities.

This model predicts that agents which change the fraction of the membrane lipids in the fluid state will of necessity change the distribution of the membrane proteins between the liquid and ordered region of the membrane lipids. If the activities of these proteins are different depending on the physical state of the lipids, then changes in the fluidity of the membrane will alter the activities of these proteins. It should be stressed that a given membrane-active agent need not produce an overall change in the order of the membrane lipids, but could act locally and transiently, affecting only a fraction of the cell membrane.

On the basis of this model we propose that control of cell growth, in general, may be dependent upon modulation in the organization of the cell membrane. In particular, we propose that differences in the growth characteristics of normal and transformed cells could be due to primary changes in membrane fluidity and structure that modulate the activity of membrane enzymes which are critical to regulation of cellular proliferation.

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