Fluorescence Photobleaching Recovery Measurements of Surface Lateral Mobilities on Normal and SV40-Transformed Mouse Fibroblasts†

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ABSTRACT: Lateral mobilities of fluorescent cell surface probes have been measured on normal (3T3) and transformed (SV3T3) cultured mouse fibroblasts. There is little discernible difference in the mobilities of a lipid analogue (diI), a fluorescent ganglioside derivative (G_M1), and tetramethylrhodamine-labeled succinylated concanavalin A. The two cell lines showed expected differences in their abilities to grow in agar, to grow without serum, and to be agglutinated by lectins, indicating that changes of these properties in transformed cells are probably not mediated through increased overall membrane fluidity but are associated with distinct alterations in the mobilities of cell surface receptors. Both fluorescent dextran derivatives and antimouse cell surface antibodies were distinctly less mobile on SV3T3 cells, and the mobile fraction of Con A receptors was lower on SV3T3 cells.

Transformation of cultured animal cells by oncogenic viruses such as SV40 induces many phenotypic changes, particularly at the cell surface. For example, SV40-transformed cells can grow in multiple layers or in agar (Todaro, 1969; McPherson, 1969); they exhibit markedly different responses to potential mitogens such as plant lectins; the cell surface compositions, particularly proteins, glycoproteins, and glycolipids, are altered (Nicolson, 1976; Burridge, 1976). It is quite possible that these phenomena are structurally interrelated. The response of transformed cells to plant lectins differs in (1) their greater agglutinability by lectins and (2) the enhanced redistribution of lectin receptors on their surfaces (Rosenblith et al., 1973; Barbarese et al., 1973; Nicolson, 1974). Thus, it is natural to ask whether these properties are a consequence of greater lectin receptor mobility on SV40-transformed cells. This study responds to this question.

Our studies use measurements of lateral mobility by the fluorescence photobleaching recovery (FPR) method in which lateral motion is detected as the migration of fluorescent molecules into a small photobleached area. Fluorescence intensity from a small laser-illuminated spot (3-5 μm^2) on the cell surface is monitored both before and directly after a brief, intense bleaching pulse of laser light on the spot which creates a region partially devoid of fluorophores. The rate of subsequent migration of unbleached fluorophores into this region from the adjoining area, monitored by the fluorescence intensity, allows the determination of a diffusion coefficient of the mobile species. FPR is thus a direct measurement of mobility over macroscopic distances.

Although many studies on lateral mobility and fluidity in transformed cell membranes have appeared recently, using several spectroscopic techniques [ESR spectroscopy (Gaffney, 1975; Lee & Scandella, 1979; Hatten et al., 1978), fluorescence polarization (Inbar et al., 1973; Nicolau et al., 1978), resonance energy transfer (Fernandez & Berlin, 1976), and excimer formation rates (Burleson et al., 1978; Edwards et al., 1976)], only fluorescence polarization (Inbar et al., 1973) has suggested greater fluidity in transformed cells while other reports show comparable or lower fluidity. Recently Kosower et al. (1978) have directly observed dramatically lower rates of spread of fluorescent label in transformed cells compared to normal lymphocytes. Nevertheless, it is widely believed that membrane fluidity is a potent modulator of receptor redistribution.

The hypothesis that receptors are more mobile in transformed cells with the consequence of greater agglutinability is supported by two lines of evidence. First, Edidin & Weiss (1974) have demonstrated that Sendai virus fused transformed heterokaryons undergo more rapid mixing of surface antigens than do normal heterokaryons. Second, cells whose surfaces have been subjected to cross-linking treatments in order to immobilize surface receptors are far less agglutinable (Rutishauser & Sachs, 1974; Rutishauser et al., 1974), suggesting a requirement for receptor mobility in agglutination. However, both types of experiments involve significant alterations of the cells before measurements are made. An advantage of the FPR method of measuring receptor mobility lies in the minimal treatment which cells undergo before measurements are performed.

In view of the previous results, there are several questions we seek to answer by our measurements of lateral mobility. (1) Is the lateral mobility of surface probes altered on transformed cells? (2) Is there a fluidization of the membrane lipids in transformed cells? (3) Is there a change in sensitivity to the drugs colcemid and cytochalasin B [which affect the cytoskeleton and mobility on the cell surface (Schlessinger et al., 1977a,b, 1976)] in transformed cells? (4) Do these results correlate with alterations in other phenotypic traits such as the abilities to grow in soft agar, to be agglutinated by lectins, and to grow without serum (Jainchill & Todaro, 1970)?

In order to assess effects of transformation upon mobility, we have carried out the photobleaching experiments on two established tissue culture lines: 3T3, of mouse fibroblastic origin, and SV3T3, a line of SV40 virus-transformed 3T3 cells. Cells from these two lines could be labeled by a variety of fluorescent surface probes. As surface probes we used rhodamine-labeled succinylconcanavalin A and concanavalin A, rhodamine-labeled Fab fragments of antibodies raised in rabbits against the leukemic mouse cell line P388, and rhodamine-labeled stearoyldextran molecules, which bind to both cellular and model membranes and can be induced to undergo

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patching (Wolf et al., 1977). The lipid analogue diI (dioctadecylindocarbocyanine iodide) was used to mimic and assess fluidity of the lipid phase of cell membranes (Fahey & Webb, 1978). We have also observed mobility of a fluorescent ganglioside derivative, Fl-G_M1.

For assurance of cells' normal and transformed phenotypes, agglutinability by lectins and other phenotypic traits were compared between the cell lines via standard methods (Todaro, 1969; Nicolson, 1974; Jainchill & Todaro, 1970).

Materials and Methods

Cells. 3T3 cells and the SV40-transformed line, SV3T3, both of Swiss origin, were obtained from E. Racker. They were grown in a medium consisting of Dulbecco's modified Eagles medium (DMEM) [obtained from Gibco] supplemented with 10% (v/v) fetal calf serum (Gibco), at 37 °C at 100% relative humidity, and at 5% CO_2. 3T3 and SV3T3 were reseeded every 3 and 4 days and maintained at subsaturation densities.

Prior to photobleaching experiments cells were washed twice with Hank's balanced salt solution (HBSS) (Gibco) and then incubated with microgram per milliliter concentrations of fluorescent probes, to be noted below, usually for 15–30 min at 37 °C. After this incubation cells were washed 3 times with HBSS and left in HBSS for the remainder of the experiment.

Labels and Reagents. Concanavalin A (Con A) was obtained from Vector Laboratories. Succinylconcanavalin A (S-Con A) was prepared according to the method of Gunther et al. (1973). Both Con A and S-Con A were conjugated with tetramethylrhodamine (TMR) isothiocyanate by reacting in 1 N NaCl–10% sucrose–0.05M carbonate buffer at pH 9.5 for 5–6 h at room temp. The reaction was stopped by dialyzing away unreacted tetramethylrhodamine isothiocyanate. The resulting products were separated on a Sephadex G-75 column according to the method of Agrawal & Goldstein (1967).

diI (dioctadecylindocarbocyanine iodide) was gift from Dr. A. Waggoner. Acetylated rhodamine-labeled stearoyldextran was a gift from Dr. P. Henkart. Anti-P388 Fab fragments were a gift from Dr. G. Edelman. (These are monovalent antibody fragments obtained from antisera raised in rabbits against the leukemic mouse cell line P388. They therefore contain Fab fragments reactive against various surface antigens.) A fluorescent ganglioside derivative fluorescein G_M1 was a gift from Dr. H. Wiegandt. Fluorescein wheat germ agglutinin was purchased from Miles Laboratories.

Concentrations of the probes used during incubations were as follows: TMR–Con A, 0.5–180 μg/mL; TMR–S-Con A, 20 μg/mL; diI, 3.3 μg/mL with 1% ethanol; TMR–anti-P388 Fab, 100 μg/mL; AcRSD, 50–100 μg/mL; Fl-G_M1, 2.5–10 μg/mL; Fl-WGA, 15–150 μg/mL.

Some experiments with TMR–S-Con A were done on drug-treated cells in order to examine involvement of cellular metabolism (sodium azide, 0.01 M, 30 min at 37 °C; 2-deoxy-glucose, 0.01M, 30 min at 37 °C). Drug incubations were also in HBSS. After incubations with drugs, cells were washed twice in fresh HBSS before incubation with TMR–S-Con A.

Photobleaching Technique. Cells in 35-mm Petri dishes in HBSS were observed through a Zeiss Universal microscope. A laser beam was focused by the microscope onto a small spot (~3–5 μm^2) on the cell membrane. Laser-induced emitted fluorescence from this spot was monitored by a photomultiplier tube. During fluorescence monitoring the laser beam was attenuated through (typically) an OD 3.9 neutral density filter. This filter could be briefly removed from the beam by a programmed solenoid to give a photobleaching pulse, short compared to times required for diffusion in or out of the spot, destroying a large fraction of fluorescing molecules in the monitoring area. The filter could be replaced immediately after the bleaching so that fluorescence recovery could then occur due to lateral transport of unbleached fluorescent molecules into the spot. The rate of recovery depends directly on the diffusion coefficient D, determined through curve-fitting procedures (Axelrod et al., 1976).

Cell Agglutinability Tests. Nearly confluent cultures of each line were treated with 1 mM Na_2EDTA to release cells from the substratum. Cells were centrifuged and resuspended in 1 mL of DMEM without serum, and 0.2-mL aliquots were added to 0.2-mL lectin solutions in DMEM, at various concentrations from 6 to 600 μg/mL, in 35-mm Petri dishes. After 30 min of incubation at 37 °C, droplets were observed under a microscope, and agglutination was scored on the number and size of cell lumps from 0 to ++++. No 37 °C incubation was necessary with wheat germ agglutinin.

Cell Growth Tests. Inocula of trypsinized cells from each line were seeded into DMEM containing 1% fetal calf serum and into serum-free media to test for growth in the absence of serum. Cultures were incubated and examined for growth over several days.

Solutions of DMEM supplemented with 10% FCS and 0.5 or 0.37% Difco Bacto agar were prepared; the 0.5% solution was poured into Petri dishes. Inocula were layered on next and then covered with the 0.37% agar medium solution. Dishes were incubated and observed daily for cell colonies.

Statistical Analysis of Data. Because moderate cell-to-cell variations in mobilities of fluorescent probes are typical in FPR experiments, it was appropriate to use statistical tests to evaluate differences between the two cell lines. Several FPR curves were obtained from each line with each probe. From these curves two parameters, the characteristic diffusion time τ_D and the degree of recovery of bleached fluorescence, denoted as f, were obtained. Application of a modified Student's t test (Brownlee, 1965) gave a measure of the distinguishability of the mean value of τ_D or f between the two cell lines as described by Maeda et al. (1979). To avoid additional uncertainties due to beam size, we performed experiments with a given probe on both cell lines during a single period in which no adjustments of the laser beam were required. Table I shows averages, standard deviations, numbers of observations for each line with each probe, and resulting values of t and its statistical probability for comparisons between lines. Each group of experiments was carried out at least twice to control for possible variations in the conditions and quality of the cell cultures.

Results

Responses of Both Cell Lines to Labeling with Fluorescent Probes Were Similar. Initial labeling of either cell line with any of the above fluorescent probes gave uniform distributions of fluorescence on the cell surface. With TMR–S-Con A, AcRSD, TMR–aP388 Fab, and Fl-G_M1, the uniformity persisted for periods up to 0.5 h and longer; it is known, however, that clusters of AcRSD fluorescence arise at longer times (Wolf, 1978). diI labeling conditions were critical in that doses and incubation times had to be found for sufficient labeling to be obtained before internalization occurred. The former required at least 5 min at 37 °C, 3.3 μg/mL, while the latter could begin as early as 7 min after incubation. Internalization was observed as the presence of large round fluorescent vesicles which were excluded from the cell nucleus. Cells labeled with either TMR–Con A or Fl-wheat germ agglutinin developed inhomogeneous surface fluorescence

Table I

Mobilities of Fluorescent Surface Probes on 3T3 and SV3T3 Cells

probe	cell	$\langle D \rangle \pm \sigma_D$	$\langle f \rangle \pm \sigma_f$	N
dil	3T3	$(8.96 \pm 0.80) \times 10^{-9}$	0.95 ± 0.07	6
dil	SV3T3	$(8.68 \pm 1.32) \times 10^{-9}$	0.94 ± 0.08	13
Fl-G_M1	3T3	$(4.97 \pm 0.73) \times 10^{-9}$	0.92 ± 0.10	16
Fl-G_M1	SV3T3	$(4.24 \pm 1.25) \times 10^{-9}$	0.89 ± 0.08	17
AcRSD	3T3	$(3.10 \pm 0.86) \times 10^{-10}$	0.75 ± 0.24	8
AcRSD	SV3T3	$(1.57 \pm 0.93) \times 10^{-10}$	0.81 ± 0.19	7
TMR-S-Con A	3T3	$(5.10 \pm 1.66) \times 10^{-10}$	0.73 ± 0.17	5
TMR-S-Con A	SV3T3	$(3.68 \pm 0.42) \times 10^{-10}$	0.45 ± 0.19	7
RaP388	3T3	$(2.4 \pm 0.54) \times 10^{-10}$	0.47 ± 0.18	17
RaP388	SV3T3	$(1.25 \pm 0.46) \times 10^{-10}$	0.36 ± 0.17	12
Con A	3T3	$(1.29 \pm 0.86) \times 10^{-10}$	0.17 ± 0.12	20
Con A	SV3T3	$(1.26 \pm 0.83) \times 10^{-10}$	0.07 ± 0.05	15

t Tests of Significant Differences in $\langle D \rangle$ and $\langle f \rangle$

probe	t for differences	parameter	probability that difference is statistical only[a]
dil	0.57	D	>0.50
Fl-G_M1	2.06	D	≈0.05
AcRSD	3.29	D	<0.010
TMR-S-Con A	1.65	D	>0.10
RaP388	5.31	D	<0.005
TMR-S-Con A	2.39	f	<0.05
TMR-Con A	3.33	f	<0.01

[a] That is, the difference is not significant.

distributions within 5–10 min after washing off unbound TMR–Con A or ~30 min after removing unbound Fl-wheat germ agglutinin.

No Differences in Membrane Fluidity Could Be Detected between 3T3 and SV3T3 Cells with dil; Fl-G_Ml May Be Slightly Less Mobile on SV3T3 Cells. In a typical experiment several FPR curves were recorded from cells labeled with dil or G_M1; diffusion coefficients and degrees of recovery of bleached fluorescence were estimated from these. Average values and standard deviations for a given probe were calculated and compared for significant differences between the two cell lines by using the t test. Table I shows that for dil standard deviations are larger than differences between averages for both diffusion coefficients and fractions of recovery. According to the t test, the probability that the observed difference is significant is less than 50%.

The mobility of Fl-G_Ml incorporated into cell membranes was measured and compared in the same manner. Here the t test indicated that the probability of a significant difference was about 95%, which is the threshold of significance in conventional test usage. It is at least safe to conclude that the moblility of this probe is not greater on SV3T3 than on 3T3 cells. No significant difference was observed in the fraction of fluorescence which recovered from photobleaching.

Mobility of Acetylated Stearoyldextran Molecules Is Significantly Less on SV3T3 Than on 3T3 Cells. Cells from both lines were incubated with AcRSD, and FPR curves were recorded. Statistical analyses showed that mobile fractions, i.e., the fraction of fluorescence which recovers from photobleaching, were similar on both lines but that this fraction recovers significantly more slowly on SV3T3, with a confidence level of better than 99%. The same results were obtained in a repeated experiment. Data from one set of experiments are shown in Table I.

Mobility of Rhodamine Anti-P388 Fab Fragments Is Significantly Less on SV3T3 Than on 3T3 Cells. Cells from each line were labeled with RaP388 Fab fragments, and several FPR curves were taken on several cells. The t test on the resulting averages and standard deviations showed that lower diffusion coefficients observed on SV3T3 cells were probably (99% or better) the result of a significant difference. However, there again did not seem to be a significant difference in the degree of recovery of bleached fluorescence. A later set of similar experiments gave similar results to data shown in Table I.

Mobility of Con A Receptors Bound Both by Con A and by S-Con A Is Similar on 3T3 and SV3T3 Cells, While the Degree of Fluorescence Recovery Is Less on SV3T3 Cells. The mobilities of TMR-labeled Con A and S-Con A on membranes of both cell lines were compared in a manner identical with that used with the above probes. Table I shows that again differences in diffusion coefficients of TMR–S-Con A between the two cell lines are obscured by variations within the cell lines; there is less than a 75% chance that the difference is significant. However, the corresponding mobile fractions are significantly less on SV3T3 cells, with better than a 95% chance of significance.

Cultures of SV3T3 and 3T3 cells were incubated with doses of TMR–Con A ranging from 5 to 180 μg/mL, and FPR curves were recorded from immediately after the incubation with the probe up to 3 h after the incubation. With these two cell lines, no trends were seen in either diffusion coefficients or mobile fractions with respect to time or to dose. Therefore, all data were combined for statistical comparisons between cell lines. Results are shown in Table I. Clearly, there is little discernible difference in the apparent diffusion coefficients, while there is significantly less total fluorescence recovery on the SV3T3 cells.

3T3 and SV3T3 Lines Show Expected Differences in Serum Dependence, Anchorage Dependence, and Agglutinability. Growth of both SV3T3 and 3T3 was observed in DMEM supplemented by fetal calf serum. Both lines could grow to confluence in 1% serum, and the SV3T3 cells grew noticeably faster. Even in media supplemented with 10% fetal calf serum, SV3T3 cells tended to grow faster. In serum-free media the density of SV3T3 cultures increased slowly, while that of 3T3 cultures remained unchanged. Cells from both lines were trypsinized and then seeded, at various densities, between layers of 0.5 and 0.3% Difco agar in Dulbecco's MEM with 10% fetal calf serum. SV3T3 cells consistently grew into large colonies after periods of 2–3 days, while 3T3 cells formed no colonies over periods of 5 days.

Cells from both lines were plated at a density of 1×10^6 per 25-cm^2 culture flask. After 24 h these were suspended by using 1 mM Na_2EDTA, centrifuged, and resuspended in 1 mL of Dulbecco's MEM without fetal calf serum. The cell suspension (0.2 mL) was added to 0.2 mL of a lectin solution (either Con A or wheat germ agglutinin) whose concentration varied from 6 to 600 μg/mL (also in DMEM without serum). Cells were observed under a microscope after 30-min incubation at 37 °C. No incubation was necessary with wheat germ agglutinin. The degree of agglutination was scored semiquantitatively from 0 to ++++ based both on the fraction of cells in clumps and on the size of clumps. For similar degrees of agglutination (++), SV3T3 required 3 μg/mL Con A and 10 μg of WGA, while 3T3 required 300 and >300 μg/mL, respectively. Thus, SV3T3 was 30–1000 times more readily agglutinated.

Discussion

Our measurements indicate that the mobility of dil is the same within experimental error on the cell membranes of 3T3 and SV40 virus transformed 3T3 cells. The mobility of dil has been shown to be a good indicator of phase transitions in lipid membrane systems (Fahey & Webb, 1978). Thus, the

transformation of 3T3 cells by SV40 virus does not cause a change in lipid fluidity which we could detect under the conditions of these experiments. This conclusion agrees with those of other studies using ESR (Gaffney, 1975; Hatten et al., 1978), fluorescence polarization of DPH (Nicolau et al., 1978), and rates of formation of pyrene excimers (Ewards et al., 1976), all of which measure mobilities or consequences thereof of small molecules incorporated into cell membranes.

The increased agglutinability of transformed cells has been taken to indicate increased membrane fluidity by supposing, first, that the rate of agglutination is controlled by the rate at which lectin receptors can migrate into regions of intercellular contact to provide stable links between cells. Therefore, increased agglutinability is supposed to reveal increased lectin receptor mobility. Second, supposing that receptor mobility is controlled by the viscosity of the membrane lipid bilayer, increased agglutinability has been taken to indicate increased lipid fluidity. Our results contradict this line of argument at two crucial points. First, although the SV40-transformed cells which we examined were more readily agglutinated by Con A than "normal" 3T3 cells, the mobility of the S-Con A receptors was not increased but was, if anything, decreased relative to that of normal cells by transformation. Hence, it appears that the lectin receptor mobility does not control the rate of agglutination. However, an alternative is suggested by the observations of Schlessinger et al. (1977b) who found that Con A receptor mobility is gradually decreased on L-6 cells on incubation with S-Con A or Con A. If this immobilization occurred during incubation of 3T3 and SV3T3 cells too rapidly to detect by our FPR measurements, it could hide an initial difference of receptor mobility. However, this scenario seems unlikely since the membrane proteins labeled nonselectively by RaP388 showed no such difference. Futhermore, *direct* measurements of diI mobility reveal no detectable change in membrane fluidity. Therefore, it seems that agglutinability need not indicate membrane fluidity and that the mechanism described above is insufficient to explain the kinetics of agglutination. Fernandez & Berlin (1976) and Jacobsen et al. (1976) have obtained results similar to ours in measurements of the mobility of Con A on surfaces of 3T3 and SV40-transformed 3T3 cells via resonance energy transfer and FPR, respectively.

Table I shows that substantial uncertainties are associated with many of our experimental results. In some instances the relatively large standard deviations listed may be due to variations from cell to cell or from one region to another on the same cell in addition to random measuring errors. Indeed, we have insufficient data to verify that our measurements are normally distributed about mean values as required for the statistical analysis (Brownlee, 1965) which we have used. Therefore, we emphasize that the conclusions which we draw must be qualified by the uncertainties in our measurements. For example, although we have detected no statistically significant change in diI or Con A receptor mobility as a result of SV40 transformation, it could be argued that changes could have occurred which were too small to detect in our measurements but which nevertheless had important effects on the rate of receptor aggregation and cell agglutination. Although we think this is unlikely, we cannot rule it out definitively without quantitative measurement of receptor aggregation and cell agglutination and a quantitative theory which relates these processes to receptor mobility.

Subject to these uncertainties, our measurements suggest that the diffusion coefficient of AcRSD and RaP388 and the fraction of mobile S-Con A receptors are decreased by SV40 transformation. This reinforces a conclusion, which we reached in earlier studies (Schlessinger et al., 1977a,b), that lipid fluidity (as measured by diI mobility and which seems to be unaffected by transformation) is not the sole determinant of membrane protein mobility. Con A and RaP388 label an uncharacterized collection of plasma membrane components. This complicates interpretation of effects on their mobility because some Con A receptors and binding antigens for RaP388 might be relatively mobile glycolipids. Furthermore, the apparent decrease in the diffusion coefficient of AcRSD demonstrates an effect of transformation on a membrane-bound synthetic lipopolysaccharide. One plausible mechanism to account for the observation that transformation reduces the mobility of various membrane components without a major decrease in lipid fluidity would be for transformation to alter the population of membrane proteins in such a way that the more mobile portion of the population is reduced.

Although we have no basis for a detailed structural interpretation of our results, it is interesting to consider whether microfilaments may play a role. Ash & Singer (1976) have found that the distribution of Con A receptors on the surface of normal rat kidney cells was correlated with myosin (and therefore presumably actin) filaments revealed by immunofluorescence below the cell surface. Pollack et al. (1975) have shown that SV40 transformation disrupts microfilaments in SV3T3 cells. In our experiments both normal and transformed cells showed similar responses to the microfilament-disrupting drug cytochalasin B. This indicates that, in spite of the effects of transformation on actin microfilaments, cytochalasin B can cause additional effects which are comparable in both normal and transformed cells in decreasing both the diffusion coefficient and mobile fraction of Con A receptors. It would be interesting then to discover whether cytochalasin B acts on actin-containing structures which are unaffected by transformation and what role these structures might play in determining receptor mobility.

We have examined three well-known phenotypic markers of transformation. As expected, the transformed cells which we studied formed colonies in soft agar, grew in low serum conditions, and were more agglutinable than their normal counterparts. One purpose of these experiments was to assure that the two cell lines retained their normal and transformed phenotypes after many passages in cell culture. Another purpose was to be able to ask whether the effects of transformation on these properties were mediated through changes in membrane fluidity or receptor mobility. We conclude that membrane fluidity, as measured by the mobility of diI, is not involved. There does, however, appear to be a correlation between changes in phenotypic characteristics and a small decrease in the mobility of some surface components on SV-transformed 3T3 cells. Whether or not this small selective decrease in mobility is seen also in other normal/transformed pairs of cell lines remains to be determined. Moreover, there is no present basis for speculation about the physiological significance of these changes in mobility. Clearly, the control of surface mobilities is a complex process since it appears to function independently of membrane fluidity and differently for different types of membrane receptors. Furthermore, we have shown that changes in agglutinability [and possibly also in lectin receptor redistribution (Rosenblith et al., 1973; Barbarese et al., 1973; Burleson et al., 1978; Edidin & Weiss, 1974)] which result from transformation cannot be simply interpreted in terms of increases in membrane fluidity and receptor mobility.

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Effect of Ethidium Bromide on Deoxyribonucleic Acid Internal Motions†

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ABSTRACT: We have used ^{31}P and ^{1}H NMR to show that DNA internal motions are stopped or greatly hindered when ethidium bromide intercalates into DNA. We show that the effect is localized to a 2 base pair long DNA region at the binding site and that internal motions outside the binding site are nearly unaffected. Based upon the nearly independent behavior of bound and free DNA regions, we conclude that in B-DNA individual base pairs experience internal motion which is independent of the motion of their neighbors.

Recent nuclear magnetic resonance studies of long DNA[1] fragments have shown that the internal structure of B-form DNA is not rigid but instead experiences large fluctuations in nucleotide conformation which occur with a time constant near 10^{-9} s (Hogan & Jardetzky, 1979; Bolton & James, 1980; Klevan et al., 1979; Early & Kearns, 1979).

Based upon ^{31}P NMR relaxation measurements, Klevan et al. (1979) have shown that the backbone phosphates of DNA

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[1] Abbreviations used: ^{13}C, carbon-13; DNA, deoxyribonucleic acid; EB, ethidium bromide; ^{1}H, proton; NMR, nuclear magnetic resonance; ^{31}P, phosphorus-31.

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