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LATERAL DIFFUSION OF MEMBRANE LIPIDS AND PROTEINS IS INCREASED SPECIFICALLY IN NEURITES OF DIFFERENTIATING NEUROBLASTOMA CELLS

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Summary

Lateral diffusion of membrane lipids and proteins was determined in differentiating C1300 mouse neuroblastoma cells by fluorescence photobleaching recovery measurements. It is demonstrated that upon differentiation the lateral diffusion of membrane lipids and proteins is increased specifically in the extending neurites. This indicates the appearance of a topographical heterogeneity in the cell membrane, whereby more fluid domains become located in the membrane of the neurites.

Dynamic properties of surface membrane components can be studied on intact cells by the fluorescence photobleaching recovery method (FPR). This method provides sufficient spatial resolution to resolve local heterogeneities within the surface membrane of a single cell [1—4]. We have applied FPR to measure the lateral diffusion of both membrane lipids and membrane proteins during the outgrowth of neurites in differentiating neuroblastoma cells. Upon differentiation these cells express to a large extent the characteristic features of mature nerve cells [5—7]. Recently de Laat et al. [8] demonstrated that the outgrowth of neurites is accompanied by a decrease in 'microviscosity' of the cell membrane lipid matrix, as derived from

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Hepes, *N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid.

fluorescence polarization measurements of a lipid probe. We now demonstrate that the lateral mobility of both membrane lipids and membrane proteins is increased specifically in the outgrowing neurites. This indicates the existence of a topographical heterogeneity in the cell membrane of differentiating neuroblastoma cells, and suggests that the majority of fluid lipid domains in the cell membrane are located in the neurites.

C 1300 mouse neuroblastoma cells (clone Neuro-2A) were cultured in 35 mm plastic petri-dishes in Dulbecco's modified Eagle's medium supplemented with 10% foetal calf serum, without bicarbonate but with 25 mM Hepes-buffer, pH 7.5. Differentiation was induced 24 h after plating by the addition of 200 μg 3-isobutyl-1-methylxanthine per ml plus 1 mM dibutyryl cyclic AMP to the medium, as described before [8]. As a fluorescent probe for the lateral diffusion of membrane lipids we used a fluorescein-labeled analogue of the ganglioside GM1 (F-GM1: a gift from H. Wiegandt). Membrane proteins were labeled with rhodamine-labeled rabbit antibodies (*Fab'* fragments) against mouse El4 lymphoid cells (RaEl4: a gift from G. Edelman), which showed cross-reactivity with surface antigens of Neuro-2A cells. After the cells were washed with Hank's balanced salt solution, they were labeled by incubation of the cells at 37°C in this solution with (i) 5 μg/ml of F-GM1 for 10 min, or (ii) 100 μg/ml of RaE14 for 30—45 min. After labeling the cells were washed several times with Hank's balanced salt solution. Separate dishes were used for the two probes. Fluorescence microscopy revealed a uniform surface labeling in all cases without any sign of internalized fluorophores.

The lateral motion of the labeled cell surface components was measured by the fluorescence photobleaching recovery method [1—4]. Fluorophores within a small area (radius about 1 μm) were irreversibly photobleached by a short pulse of intense focused laser light. Rates of lateral diffusion were calculated from the recovery of fluorescence in the bleached region due to the entry of unbleached fluorophores from adjacent parts of the membrane, measured with 1/1000 attenuated fluorescence excitation (λ = 488 nm for F-GM1; λ = 514.5 nm for RaEl4). The fluorescence recovered as expected for a single diffusion coefficient [1]. Diffusion was characterized by lateral diffusion coefficients, D (cm^2/s). The fractional recovery of fluorescence after bleaching was taken as a measure for the fraction of mobile fluorophores on the time scale of our measurements. Routinely, fluorescence bleaching recovery curves were determined for each cell from at least two surface locations in the perikaryon region and from two locations along the longitudinal axis of the outgrowing neurite: one half way and one at the tip (see Fig. 1). For each location two successive bleaches were analyzed. Most measurements were taken after 24 h of differentiation, at which time extensive neurites of 100—200 μm have been formed (Fig. 1). A number of fluorescence bleaching recovery curves were recorded for F-GM1 also after 48 h. Care was taken to select for the measurements cells which had developed neurites of sufficiently large diameter (greater than 5 μm) in comparison to the diameter of the bleached region, to exclude significant deviations from the assumed two-dimensional diffusion. In no case was any significant difference observed between measurements at

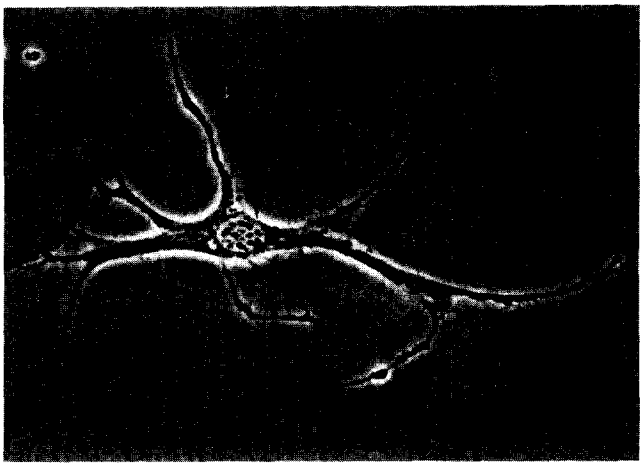

Fig. 1. Phase micrograph of a Neuro-2A cell after 24 h of differentiation. Arrows indicate typical locations where the laser beam was focused and fluorescence photobleaching recovery recordings were made: (a) perikaryon region; (b) neurite region; (c) tip of the outgrowing neurite.

different locations along the neurite. We therefore compared the lateral mobilities in the perikaryon region with those in the neurite. For F-GM1 the fractional recoveries were nearly 100% in all cases, indicating the absence of immobilized lipid probe molecules. RaEl4 showed 30—40% recoveries independent of the location at the cell surface. Apparently about two-thirds of the labeled proteins were immobile on the measured time scale.

The measured diffusion coefficients are summarized in Table I and an analysis of variance [9] was performed on the data. After 24 h of differentiation, the lateral mobilities of F-GM1 and RaEl4 in the perikaryon region were not significantly different from those of undifferentiated cells ($P>0.5$ in both cases), and comparable to those observed in interphase cells in synchronized cultures of the same type of cell [10]. However, in the outgrowing neurites the diffusion coefficients of F-GM1 and RaEl4 were significantly enhanced as compared to the perikaryon ($P<0.005$ and $P<0.01$, respectively). Upon prolonged differentiation F-GM1 showed an even more pronounced increase in lateral mobility in the neurite region ($P<0.001$).

TABLE I

Lateral diffusion coefficients of membrane lipids (F-GM1) and membrane proteins (RaEl4) in the perikaryon region and in the outgrowing neurite of differentiating Neuro-2A cells. The values for the perikaryon region of undifferentiated cells (control) were taken from synchronized cells at the transition from the G1- to the S-phase. The results are given as mean ±S.E. (number of independent measurements). n.d., no data.

Time of differentiation	F-GM1 (10^{-9} cm^2/s)		RaEl4 (10^{-10} cm^2/s)	
	Perikaryon	Neurite	Perikaryon	Neurite
Control	5.7±0.4 [10]	—	2.1±0.3 [10]	—
24 h	5.3±0.4 [11]	7.1±0.3 [25]	1.8±0.4 [10]	3.5±0.5 [18]
48 h	6.5±0.4 [10]	9.9±0.5 [17]	n.d.	

Our results demonstrate that the lateral diffusion of both membrane lipids and proteins are specifically increased in the outgrowing neurites. This demonstrates a topographical heterogeneity in the cell membrane of differentiating neuroblastoma cells. Our data show also that the relative increase in protein mobility after 24 h differentiation is larger than the change measured for the lipid probe (see Table I). This could indicate that the motion of the lipids and proteins in the cell membrane are at least partially governed by different constraints, which is consistent with our observation that the lateral diffusion of surface lipids and proteins is modulated independently through the greater part of the cell cycle [10]. Our current results are qualitatively consistent with the earlier fluorescence polarization [8] and spin labeling [11] studies, showing a decrease in 'microviscosity' and increase in membrane fluidity, respectively. Furthermore, they are consistent with the reported increase in the mobility of concanavalin A receptors in differentiating neuroblastoma cells [12]. A similar qualitative correlation between the 'microviscosity' and the lateral diffusion of membrane lipids was found during the cell cycle of neuroblastoma cells [10,13]. We have shown furthermore that the newly formed membrane incorporated in the outgrowing neurites has dynamic properties different from those of the surface membrane of the perikaryon. This may indicate that there is a relative high concentration of more fluid lipid domains in the neurite. It remains to be shown whether this is a necessary condition for the cell to express its functional neuronal membrane properties upon differentiation.

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