Effect of Microvilli on Lateral Diffusion Measurements Made by the Fluorescence Photobleaching Recovery Technique

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EFFECT OF MICROVILLI ON LATERAL DIFFUSION MEASUREMENTS MADE BY THE FLUORESCENCE PHOTOBLEACHING RECOVERY TECHNIQUE

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ABSTRACT To consider the effect of surface microvilli on measurements of lateral diffusion by fluorescence photobleaching recovery, we have measured the diffusion of the lipid probe 3,3'-dihexadecyl indocarbocyanine iodide on the villated main body and unvillated budding polar body of unfertilized mouse eggs. On the main body we found $D = (6.41 \pm 0.62) \times 10^{-9}$ cm^2/s with (77.0 ± 2.1) % recovery, and on the budding polar body we found $D = (7.05 \pm 0.75) \times 10^{-9}$ cm^2/s with (84.7 ± 1.3) % recovery. We thus find only slight differences in diffusion in the two regions.

INTRODUCTION

The technique of fluorescence photobleaching recovery (FPR) has been extensively used to measure the lateral diffusion of membrane components on cell and model membranes (for reviews, see references 1, 2). This technique consists in fluorescently tagging the molecule whose motion is to be studied, bleaching the fluorescence in a small spot on the membrane using a focused laser beam, and then monitoring the recovery of fluorescence by diffusion (3). Since structure of the cell plasma is generally not flat, but rather contains various microvilli and invaginations, the question has been raised whether these deviations from flatness will greatly affect diffusion measurements made by FPR (4–6). A study made by Dragsten et al. (4) on lymphocytes indicates that cell surface irregularities do not affect diffusion measurements. In cases of more extreme microvillation such as squid photoreceptor membranes considerable deviation can exist between actual and measured values of the diffusion coefficient (5). The most significant question is not of the accuracy of absolute diffusion values; rather it is of the validity of comparisons of two cell types or states.

The unfertilized mouse egg affords an excellent system for considering the effect of microvillation on FPR measurements. As is shown below in Fig. 1, this cell is not round but rather has a nipple, which is due to the budding of the second polar body. Scanning electron micrographic studies of these eggs (7) indicate that while the budding polar body has a smooth membrane devoid of microvilli, the main body of the egg has a membrane dense in microvilli. This difference in density of microvilli is reflected in Fig. 1 in the fluorescence intensity differences of the lipid-soluble dye 3,3'-dihexadecylindo carbocyanine iodide (C_{16}diI) in the two regions. The unfertilized mouse egg affords an experimental system to compare diffusion of a single probe on unvillated and highly villated regions of a continuous plasma membrane.

MATERIALS AND METHODS

Supply of Eggs

Unfertilized eggs were obtained from the ampullae of superovulated CD-1 (Charles River) female mice as described elsewhere (9). Cumulus cells were removed by minimal treatment (5–10 min room temperature) with 0.1% hyaluronidase (ICN Pharmaceuticals, Inc., Cleveland, OH) in Hanks' balanced salt solution (HBSS) buffered to pH 7.4 with HEPES and containing 0.4% BSA (HHBSS + 0.4% BSA), followed by extensive washing in HHBSS + 0.4% BSA. *Zona pellucidae* were removed by minimal incubations (15–30 s) in acid Tyrode solution (pH 2.5, 0.4% polyvinylpyrrolidone) at room temperature, again followed by extensive washing in HHBSS + 0.4% BSA.

C_{16}diI Labeling

Eggs were labeled with C_{16}diI by incubation for 8 min at 23°C in HHBSS + 0.4% BSA containing 20 μg/ml of C_{16}diI and 2% ethanol. Labeled cells were washed extensively in HHBSS + 0.4% BSA and then taken up in 100 μm pathlength microslides (Vitro Dynamics, Rockaway,

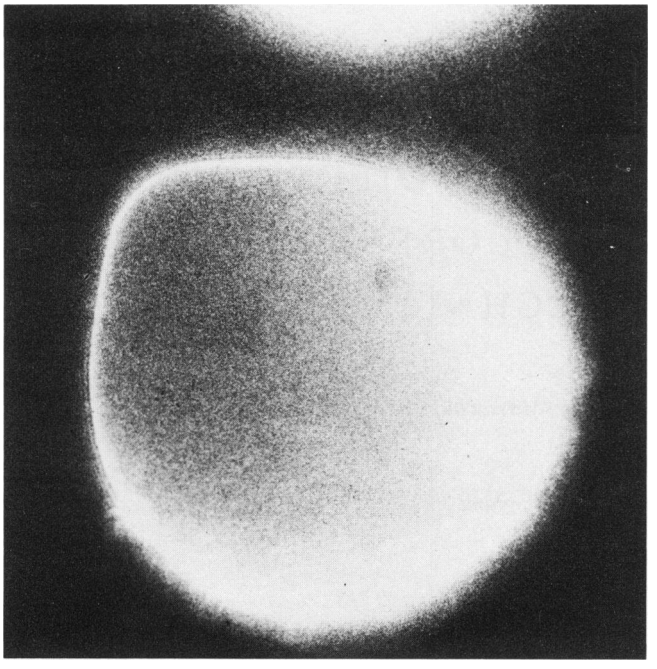

FIGURE 1 Fluorescence photomicrograph of an unfertilized mouse egg labeled with C_{16}diI. (Such differences have also been found by staining with concanavalin A [8]). The otherwise round cell shows a nipple where the second polar body is forming. Scanning electron micrographs show this nipple to be smooth and largely devoid of microvilli, whereas the round region of the egg is highly villated (7). This difference is indicated here by the diminished fluorescence in the nipple as compared with the rest of the egg, which results from the nipple's lesser surface area. This photograph is meant to indicate the qualitative nature of the difference. The print has been overexposed on high contrast paper to clearly show the ring stain of the budding polar body. This exposure is excessive for the bright fluorescence on the rest of the cell.

NJ) for examination in the fluorescence microscope or for FPR measurements. The staining pattern is shown in Fig. 1.

FPR Measurements

Our apparatus has been described elsewhere (8). We used the 531-nm laser line focused to an exp (-2) radius of $(1.1 + 0.2)$ μm. Monitoring intensities were 3 μW and bleaching times were 30 ms. Measurements were made at 23°C. Data was analyzed by the half-time method of Axelrod et al. (3).

RESULTS AND DISCUSSION

Measurements were made of C_{16}diI diffusion on the round villated and budding unvillated regions of 22 eggs. Diffusion coefficients on the villated region were $D = (6.41 \pm 0.62) \times 10^{-9}$ cm^2/s (mean ± standard error of the mean), and percentage recoveries were % Rec = (77.0 ± 2.1). On the unvillated regions we found $D = (7.05 \pm 0.75) \times 10^{-9}$ cm^2/s and % Rec = (84.7 ± 1.3). Diffusion coefficients for C_{16}dI villated and unvillated regions did not differ significantly whether compared by an unpaired or a paired Student's t test. $t = 0.68$ in the unpaired test and $t = 0.90$ in the paired test. Neither of these values is significant. Comparison of % Rec unpaired gives $t = 3.14$ and paired gives $t = 3.66$. Both values are significant at $P < 0.01$.

Intuitively we might expect that molecules would take longest to reach the tip of the microvilli and that this would show up empirically as a lower mobile fraction. The effect we have observed is consistent with this interpretation.

The results suggest the quantitative limits on comparative studies made by FPR. We have performed a similar series of experiments on cell pairs of 16-cell mouse embryos. One member of each pair has significantly more microvilli than the other but we have not detected any significant differences in D or % Rec for the even n $C_n$diI (n = 14 to 22; data tested in a paired t test; to be published elsewhere).

An idea of the size and density of microvilli in this experiment can be obtained from Eager et al. (7; Figs. 1 and 2.) The difference in the number of microvilli in the two regions of the unfertilized egg is large. Essentially we are comparing a region devoid of microvilli to a region dense in microvilli. Even under these extreme conditions no large differences in diffusion were observed. The majority of FPR studies to date have dealt with systems where considerably smaller differences in density of microvilli occur. Hence such differences are not likely to be responsible for the observed variation in diffusion coefficients. An attempt to treat this problem theoretically may be found in the accompanying paper by Aizenbud and Gershon (10).

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