RESTRICTED LATERAL DIFFUSION OF SURFACE MEMBRANE COMPONENTS IN *TETRAHYMENA THERMOPHILA*

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Received 27 April 1981

1. Introduction

The mechanisms whereby proteins are spatially oriented in the membrane bilayer remain the object of intense speculation. Since membrane proteins would be expected to diffuse rapidly in the lateral plane unless restricted [1], experiments have concentrated on determining how the lateral movement of proteins is controlled, and in particular whether this could occur by specific binding to cytoskeletal elements.

If the lateral mobility of proteins is controlled by binding to cytoskeletal elements, then variations in the lateral mobility would not be expected to occur if the membrane fluidity were changed isothermally. A mutant auxotrophe of *Tetrahymena thermophila*, an organism with a highly organised cortical morphology [2], has been isolated. In this mutant the membrane fluidity can be altered over a wide range by supplementation with various fatty acids [3]. Before examining the lateral mobility of surface components of the mutant, it was necessary to study the wild-type organism, and establish whether variations in the lateral mobility occurred under normal experimental conditions.

Reported here are fluorescence photo-bleaching recovery (FPR) measurements which demonstrate that a large fraction of the surface proteins are immobile, both when intact antibodies and monovalent Fab fragments are used as probe. However, starvation of cells leads to an increase in the fraction of mobile surface components. Furthermore, there are indications that, as in other cell systems [4,5], variations in the lateral diffusion occur during the cell cycle.

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2. Materials and methods

Antibodies to surface components of *T. thermophila* were prepared by immunising rabbits subscapularly with an emulsion of pellicles (prepared according to [6]) in complete Freunds adjuvant. Boosting injections were administered in weeks 3 and 7. After bleeding in week 8, the gamma glubulin (γG) fraction of the antiserum was precipitated using ammonium sulphate [7]. Fab fragments were prepared from the γG fraction by digestion with papain (Worthington) following which the digest was dialysed overnight at 4°C against water. The white precipitate was centrifuged off and the supernatant dialysed against phosphate-buffered saline (PBS) before passing through a Sepharose 4B–protein A column to remove Fc fragments [8]. The γG fraction or Fab fragments were then conjugated with fluorescein isothiocyanate (FITC) isomer I (Sigma) at pH 9.0 for 30 min at room temperature, excess FITC removed on a Sephadex G-25 column, and the FITC–Fab then dialysed extensively against PBS. Small aliquots were kept frozen at −80°C until use.

Tetrahymena thermophila strain B, mating type IV, were cultured at 22°C in 1% Oxoid bacterial peptone with 0.1% Oxoid yeast extract and 36 μM $FeCl_3 \cdot 6\,H_2O$. The cells were harvested at 700 × *g* and washed in an inorganic buffer with added glucose as in [9] and labelled either with FITC–γG or FITC–Fab for 10 min at room temperature. The cells were then washed 3 times in inorganic buffer, immobilised by addition of a few mg/ml dry ice [10] and examined under the microscope of the FPR apparatus. Only viable, non-aggregated and undamaged cells were chosen for FPR experiments. (Non-viable cells are easily recognised by the absence of ciliary movement, an inactive contractile vacuole and the appearance of internal fluores-

cence.) Control staining with non-specific antisera (FITC rabbit anti-mouse antiserum) showed no surface staining.

The FPR measurements were performed in the laboratory of Dr J. Schlessinger at the Weizmann Institute, Rehovot. The apparatus for FPR measurements has been described [11,12]. Photo-bleaching was typically for 0.3–0.5 s, and the measurements at 21–23°C. The $1/e$ radius of the laser beam was 1.5 μm.

3. Results and discussion

Tetrahymena is usually highly motile, but under the conditions of labelling most cells were sessile, in particular the FITC–γG antibody rapidly and effectively immobilised the cells. In cells freshly labelled with intact FITC–γG an intense peripheral staining was observed evenly distributed both on and between the ciliary rows. After ~60–90 min the label began to assume a patchy appearance. In contrast, after staining with the FITC–Fab preparation the fluorescence remained evenly distributed, even 2 h after labelling.

For determination of the lateral diffusion coefficient, the laser beam was focussed between the ciliary rows. The recovery curves were of such a shape as to preclude membrane flow or cell motility [11].

As seen in table 1 (a) the lateral diffusion coefficient for cells labelled with FITC–γG was $<10^{-12}$ cm^2/s, and the recovery was negligable, showing that the surface proteins are immobile in the presence of the bivalent antibody. However, with monovalent FITC–Fab fragments there were moderately large recoveries, showing that many of the surface components are normally mobile.

Results of 3 separate sets of experiments on randomly selected cells are presented in table 1 (b–d1). Not surprisingly, the mobility of surface components was ⩾100-fold higher than that seen with FITC–γG. The value of D in group (d1) is somewhat higher than for groups (b,c) and it was noted that this group included measurements of 3 cells labelled 5–10 min before division (easily recognised by their dumb-bell shape). The surface proteins in these 3 cells were more motile (table 1) (d3), a finding which was later confirmed by measurements on other cells. No differences in fractional recovery (R) were seen between the dividing and non-dividing cells. When the values for the dividing cells were excluded from data set (d1), it was seen that D was 7.1×10^{-10} cm^2/s (table 1) (d2), in good agreement with the other two data sets. The mean value of measurements on 22 non-dividing cells (table 1) (e) is 6.5×10^{-10} cm^2/s. These values are similar to those reported for unspecifically labelled membrane proteins [13].

The finding that D is higher just before division is interesting as in [4] D was low in mouse neuroblastoma cells during mitosis and highest at G1. Since the FPR method is very sensitive to the topography of the cell, it is important to note that the measurements in *Tetrahymena* were always carried out between the ciliary rows where the membrane is flat. Unlike other cells where the membrane surface (e.g., number of microvilli) can change markedly over the cell cycle [14] *Tetrahymena* has a very well defined and constant surface morphology [2]. Under normal growth conditions the number of ciliary meridians is constant for a given species [15] and the formation and growth of cilia takes place throughout the cell cycle [16].

In *Tetrahymena*, ~50% of the increase in cell volume/cell generation occurs over the last 15% of

Table 1

Exp. no.	Surface label	R (%)	$D \times 10^{-10}$ (cm^2/s)	n	Cell type
a	FITC–γG	<3	<0.01	12	Non-dividing
b	FITC–Fab	20.7 ± 5.1	5.8 ± 1.3	9	Non-dividing
c	FITC–Fab	21.7 ± 6.5	7.3 ± 1.9	6	Non-dividing
d1	FITC–Fab	33.9 ± 6.2	8.9 ± 2.1	7	Non-dividing
d2	FITC–Fab	36.8 ± 6.2	7.1 ± 0.7	4	Non-dividing
d3	FITC–Fab	30.1 ± 3.7	11.2 ± 0.1	3	Dividing
e	FITC–Fab	23.9 ± 9.2	6.5 ± 1.5	22	Non-dividing
f	FITC–Fab	61.1 ± 14.1	6.2 ± 1.9	4	Starved

the cell cycle [17]. This would imply a concomitant increase in surface area during cytokinesis [14]. Since there is no marked change in the ciliary number over the same period [16] the extra surface membrane cannot be generated by a resorption of ciliary membrane in a mode analogous to that seen with microvilli [14]. It is therefore surprising to see that ^{32}P incorporation into the pellicle (outer membrane system) is uniform throughout the cycle [18] suggesting that there is no burst in membrane synthesis, but rather that pre-synthesised material is introduced into the expanding membrane during cytokinesis. Whatever the explanation, it seems that the higher values of D seen in dividing cells are real; we are now studying variations in D over the whole cell cycle.

A furthur finding of interest was that maintaining the cells in inorganic medium for 2.5 h prior to the staining procedure induces an increase in the proportion of motile surface components from ~35% in non-starved cells to ~60% in the starved cells (table 1) (f). In contrast, non-starved cells, once labelled in inorganic medium showed no changes in mobility or lateral diffusion rate with time.

These changes in the anchorage of surface components under starvation conditions are of particular interest, as starvation is a prerequisite for conjugation, during which process cell fusion of the conjugates occurs. During starvation, changes in the pattern of protein synthesis occur [19] and concanavalin A binding proteins migrate to form a ring on the top of the cell marking the area of cell fusion [20]. Little else is known about the changes occuring in the surface membrane during conjugation, although it has been suggested that changes in the membrane fluidity are important [21]. Especially during the first 4 h of starvation in non-nutrient medium there is a large increase in the relative proportion of unsaturated fatty acids [22]. Such changes would be expected to give rise to alterations in membrane fluidity, as [21], and this in turn could affect the dynamic behaviour of the intrinsic membrane proteins.

Acknowledgements

This work was carried out while R. J. H. was a recipient of an EMBO long term fellowship at the Department of Membrane Research, The Weizmann Institute of Science, Rehovot. I would like to thank Professor Carlos Gitler for his numerous helpful discussions and for his support during my stay at the Weizmann Institute. At the same time I would like to express my deep appreciation to Dr J. Schlessinger for allowing me free use of the FPR instrument.

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