Biochimica et Biophysica Acta, 731 (1983) 121–126
Elsevier

BBA 71667

FUSION AND BIOCHEMICAL EXPRESSION OF MEMBRANE RECEPTORS IN FOREIGN LIVING CELLS

KRISHNA BALAKRISHNAN *, J. TODD LEWIS **, S. QASIM MEHDI and HARDEN M. McCONNELL ***

Stauffer Laboratory for Physical Chemistry, Stanford University, Stanford, CA 94305 (U.S.A.)

(Received January 12th, 1983)

Key words: Membrane fusion; Cell-surface receptor; Fluorescence microscopy; Lateral diffusion; Histocompatibility; Antigen; Cytotoxicity

Successful transplantation of cell surface molecules from the membranes of one cell type to recipient cells of a different type is described. Plasma membranes purified from donor cells were fluoresceinated and fused to recipient cells using poly(ethylene glycol) and the fate of the transplanted membrane components was followed by fluorescence microscopy. In approximately 100 min the 'foreign' membrane components were seen to cluster and internalise. During this time, judged by the criteria of hormonal stimulation and immune cytotoxic killing, the cell surface of the recipient cell mimicked the cell surface phenotype of the donor cell.

Introduction

Cell membranes are involved in functions that vary from changes in permeability and transport to mitogenesis, hormone-receptor and cell-cell interactions.

Chemical messengers (ligands) such as trophic hormones are produced by specific glands and help to communicate with, and coordinate, the activities of the cells of target organs. The initial event in the action of hormones (such as glucagon, thyrotropin (TSH), etc.) is the recognition of and binding to specific receptors on the target cell membrane. This binding leads to a transmembrane signalling event, the formation of cAMP [1] and a cascade of other biochemical reactions.

According to the mobile receptor theory [2] hormone receptors and adenylate cyclase are discrete separate entities. Only after the hormone binds to the receptor does the latter assume special properties including 'coupling' with other membrane structures which activate adenylate cyclase and lead to an increased intracellular production of cAMP. This suggestion is supported by the observation that an adenylate cyclase molecule may be responsive to more than one hormone receptor on the membrane of the same cell surface [3]. Further, Schramm [4] transferred the glucagon receptor by fusion of liver membranes into Friends erythroleukemia (Fc) cells. Membranes isolated from the recipient cells showed that the adenylate cyclase of the recipient cells could be coupled to the transplanted hormone receptor. The fate of the fused receptor was not followed in the living recipient cell.

Poste and Nicholson [5] have subsequently shown that cell surface antigens can also be transferred by fusion of membranes from lymphocyte sensitive to lymphocyte resistant cells rendering the latter susceptible to recognition and killing by immune lymphocytes.

* Present address: DNAX Research Institute for Molecular and Cellular Biology, Palo Alto, CA 94304, U.S.A.
** Present address: Immunology Branch, National Cancer Institute, Bethesda, MD 20205, U.S.A.
*** To whom correspondence should be addressed.

Abbreviations: FITC, fluorescein isothiocyanate; FRAPP, fluorescence recovery after pattern photobleaching; Gpp[NH]p, guanylyl imidodiphosphate; TSI, thyroid-stimulating immunoglobulin.

Prujansky-Jakobovits and co-workers [6] co-reconstituted solubilised lymphocyte plasma membranes and Sendai virus envelopes into vesicles. In an elegant series of experiments, they showed that the reconstituted vesicles possessed the ability to fuse with mouse lymphocytes and modify the cell surface properties of the fully viable recipient cells.

In the present study we have transferred hormone (thyrotropin) receptors and histocompatibility antigens into foreign living cells. This work describes the fate and the functional, biophysical and immunological properties of the 'transplanted' receptors in the living recipient cells.

Materials and Methods

Animals, cells and tissues. Human thyroids were obtained at operation and processed within 30 min of removal.

Balb/c and C57BL/6 mice were obtained from the Department of Radiology, Stanford University.

EL4 (H-2^b) and LSTRA (H-2^d) tumor cell lines were maintained in ascites form by intraperitoneal innoculation of C57BL/6 and Balb/c mice, respectively. C.1.18 (H-2^k) cells were grown in RPMI 1640 medium containing 10% fetal calf serum and antibiotics.

Preparation of membranes. The preparation of plasma membranes from cells and tissues has been described in detail in the accompanying paper [7]. To destroy the adenylate cyclase activity of human thyroid membranes they were treated with *N*-ethylmaleimide as described by Schramm [4].

Fluoresceination of membrane proteins. Membranes were resuspended in 0.25 M carbonate-bicarbonate buffer, pH 9.2 at a concentration of 4.0 mg membrane protein per ml. Equal volumes of fluorescein isothiocyanate (FITC) in the same buffer (1.0 mg/ml) were then added and mixed overnight at 4°C. The membranes were pelleted by centrifugation at $100000 \times g$ for 15 min and washed three times by centrifugation and resuspension in phosphate-buffered saline, pH 7.4.

Fusion of membranes into cells. Cells ($5 \cdot 10^7$) were centrifuged in polyallomer tubes at $600 \times g$ for 5 min. Membranes (1 mg protein) in 20–40 µl of RPMI 1640 were added to the cell pellet, mixed carefully by gentle vortexing and kept in a water bath at 37°C. All subsequent operations were at this temperature unless stated otherwise. One ml poly(ethylene glycol) 1500 (PEG 1500, 46% w/v in RPMI 1640, previously autoclaved for 15 min) was added with gentle and continuous mixing over 1 min. Then 10 ml of RPMI 1640 was added with gentle mixing at an approximate rate of 1 ml/min. This mixture was layered over 5 ml of 10% sucrose and 10% bovine serum albumin in RPMI 1640. After centrifugation for 10 min at $1000 \times g$, the pelleted cells were resuspended in the appropriate medium or buffer as indicated for the individual experiment. After fusion, cells were examined for viability by Trypan blue exclusion. Preparations showing less than 90% viability were discarded.

Generation of effector lymphocytes. Effector cells were generated in mixed lymphocyte culture. Spleen cells (10^7) were cultured in 5 ml of RPMI 1640 containing 15% FCS with stimulator spleen cells (10^7) that had been treated with 40 µg/ml mitomycin C for 1 h. After 4 days in culture, cells were harvested and tested for cytotoxic activity. Control cells, cultured in the absence of stimulator spleen cells, showed no cytotoxic activity.

Measurement of adenylate cyclase activity. Adenylate cyclase activity was measured by the amount of cyclic AMP (cAMP) produced by the cells [8]. Because of the small number of cells used in these studies, the amount of cAMP formed was measured by a femtomole sensitive radioimmunoassay according to the method of Harper and Brooker [9]. Thyroid-stimulating hormone (thyrotropin), thyroid-stimulating immunoglobulin (TSI), NaF and guanylyl imidodiphosphate (Gpp[NH]p) were added as adenylate cyclase stimulators at the concentrations indicated for the individual experiment.

Measurement of lateral diffusion and fluorescence microscopy. Diffusion of fluorescent membrane proteins was measured by the fluorescence recovery after pattern photobleaching (FRAPP) technique as described in detail [10,11]. The apparatus used for photography of fluorescent cells has been described [12]. A Zeiss Photomicroscope III fitted with Ni-Tec image intensifier and attached to a Nikon EL-2 35 mm camera was used. Kodak 2475 recording film was processed at ASA 3200.

Cytotoxicity assay. Cell killing was measured by

TABLE I

THE EFFECT OF POLY(ETHYLENE GLYCOL) (PEG) TREATMENT ON THE ADENYLATE CYCLASE ACTIVITY OF RECIPIENT CELLS

Thyroid stimulators were added at the concentrations indicated. Basal activity was measured in the absence of stimulators. After 30 min the cAMP formed was extracted from cells by treatment with 5% trichloroacetic acid (final concentration) and measured by radioimmunoassay as described in Ref. 8. The values for cAMP formed are mean ± S.E. of triplicates. TSH, thyrotropin.

Stimulator	pmol cAMP/30 min	
	Control cells	PEG-treated cells
None (basal)	0.39 ± 0.08	0.34 ± 0.02
NaF (10 mM)	12.30 ± 0.07	10.10 ± 0.19
Gpp[NH]p (100 μM)	18.10 ± 0.23	16.10 ± 0.38
TSH (100 mU)	0.31 ± 0.11	0.30 ± 0.12
TSI (500 μg)	0.34 ± 0.07	0.32 ± 0.11

the ^{51}Cr release assay as described in the accompanying paper [7]. Specific details are provided for the individual experiment.

TABLE II

ADENYLATE CYCLASE ACTIVITY IN RECIPIENT CELLS FOLLOWING FUSION OF HUMAN THYROID MEMBRANES

The experimental procedure was the same as that for Table I except that C.11.18 cells were treated with poly(ethylene glycol) in the presence of 1 mg human thyroid membranes for fusion. TSH, thyrotropin.

Stimulator	pmol cAMP/30 min
None (basal)	0.41 ± 0.21
NaF (10 mM)	10.10 ± 0.33
Gpp[NH]p (100 μM)	15.30 ± 0.10
TSH (100 mU)	4.50 ± 0.07
(500 mU)	6.80 ± 0.16
(1000 mU)	7.10 ± 0.10
TSI (500 μg)	8.70 ± 0.14

Results

The effect of poly(ethylene glycol) treatment on cell viability and adenylate cyclase activity

To determine the effect of poly(ethylene glycol) treatment on cell viability and adenylate cyclase

TABLE III

IMMUNE CYTOLYSIS OF CELLS AFTER FUSION OF MEMBRANES CONTAINING DIFFERENT H-2 ALLOTYPES

Fusion of LSTRA or EL4 membranes into C.1.18 cells was as described in Materials and Methods. Cells allosensitised in mixed lymphocyte cultures were incubated with the targets cells in the effector/target (E/T) ratios indicated above. Chromium release was measured after 3 h. Target cells showed greater than 90% viability following poly(ethylene glycol) (PEG) treatment or membrane fusion. Cytotoxicity is expressed as follows.

$$\text{Cytotoxicity} = \frac{^{51}\text{Cr released in the presence of effector cells} - \text{background}}{\text{Total } ^{51}\text{Cr releasable by detergent} - \text{background}} \times 100$$

The % ^{51}Cr released values are averages of quadruplets and have been rounded off to the nearest whole number for comparison. Corrections were made for spontaneous chromium release (background) by incubating target cells in the absence of lymphocytes.

Lymphocytes	E/T ratio	% ^{51}Cr released					
		Targets used					
		C.1.18	PEG treated C.1.18	EL4	C.1.18 + EL4 memb.	LSTRA	C.1.18 + LSTRA memb.
C57BL/6 (H-2^b)	10	0	2	2	3	15	8
sensitised to	20	2	4	1	0	25	20
LSTRA (H-2^d)	50	0	2	2	2	48	28
Balb/c (H-2^d)	10	2	2	22	8	2	0
sensitised to	20	0	2	39	23	1	3
EL-4 (H-2^b)	50	2	2	55	44	2	0

activity, cells were treated with the fusogenic agent in the absence of added plasma membranes. Treatment of cells with poly(ethylene glycol) did not appear to alter the enzymatic activity significantly whether stimulation was with NaF or Gpp[NH]p (Table I). It should be stressed that the subsequent viability of the recipient cells is adversely affected if contact with the fusogen exceeds one min.

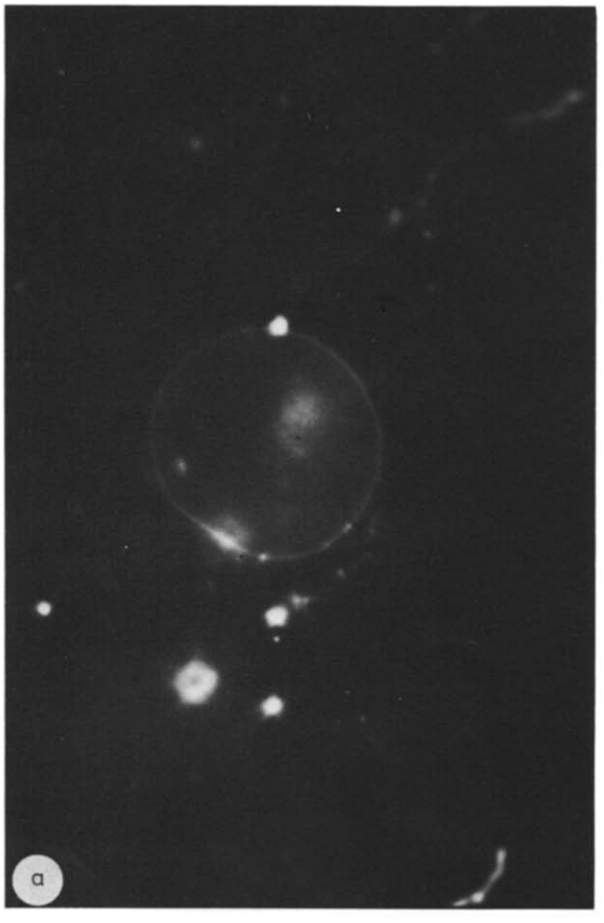
The adenylate cyclase activity of the recipient C.1.18 cells was examined in response to the thyroid stimulators thyrotropin and TSI. Basal enzyme activity was not found to increase in the presence of large excesses of both these stimulators.

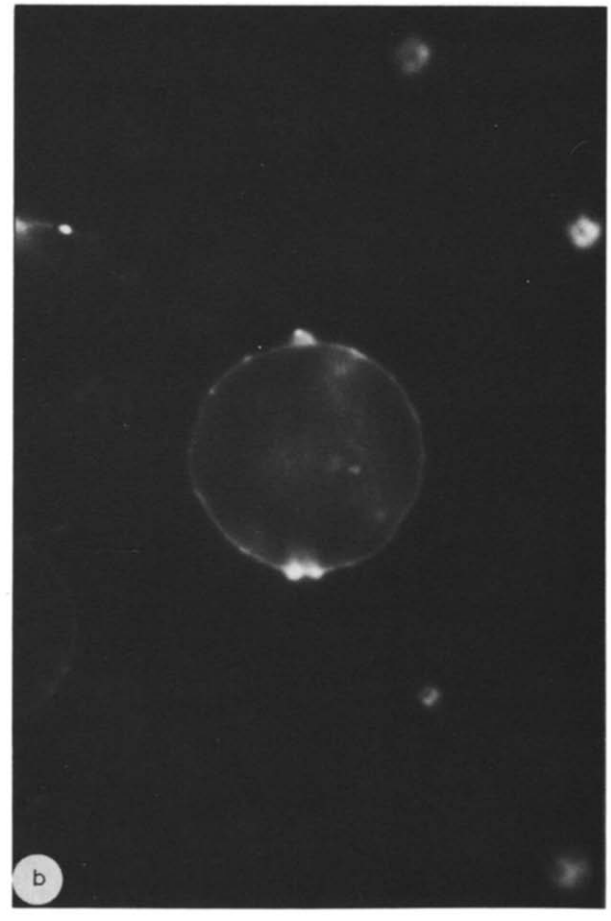
Hormonal response of recipient cells after transplantation of human thyroid membranes

When human thyroid membranes were fused into the recipient cells, thyrotropin and TSI were both found to stimulate enzyme activity in the recipient cells. It was interesting to note that this stimulation was elicited if the stimulators were presented to the fused cells within approx. 150 min after fusion and not beyond that time (Table II). The time period following membrane fusion during which the recipient cell was responsive to hormonal stimulation varied from recipient cell to recipient cell and was found to be considerably shorter (60–100 min) in adherent cells compared to those grown in suspension (data not shown).

Fusion of H-2^b and H-2^d haplotype membranes into H-2^k cells

Membranes obtained from EL4 (H-2^b) or LSTRA (H-2^d) cells were fused into C1.18 (H-2^k) cells that had been previously loaded with ^{51}Cr. To





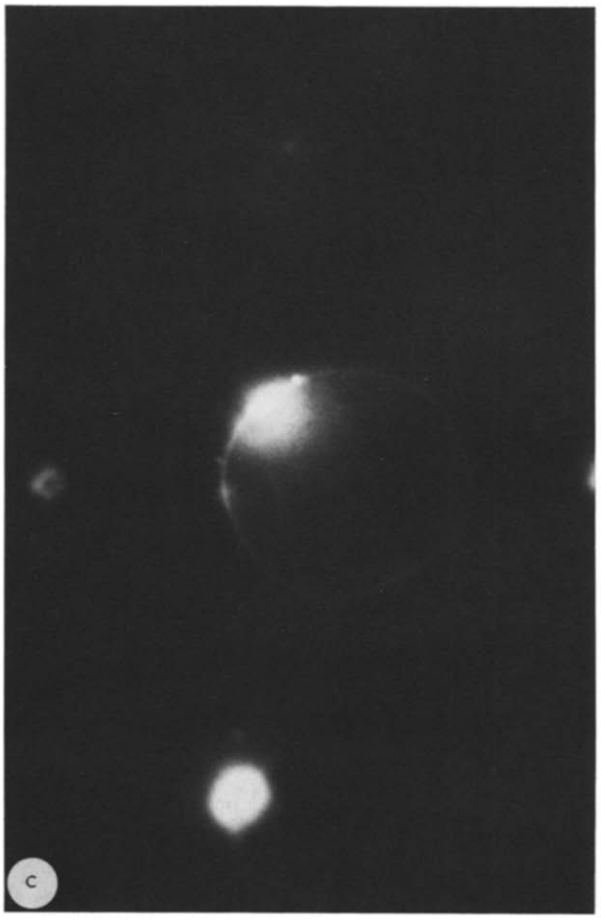


Fig. 1. Fluorescence photomicrographs of C.1.18 cells. (a) Immediately following the fusion of fluoresceinated EL4 membranes but before centrifugation to remove unfused membranes. (b) After removal of unfused membranes. (c) 120 min after membrane fusion, showing clustering of the fused fluorescent membranes. Magnification is approximately 300×.

test the susceptibility of the recipient cells to killing by cells that had been sensitised previously to H-2^b (or H-2^d) in mixed lymphocyte culture, the recipient cells were tested in the cytotoxicity assay at various effector to target ratios. Untreated C1.18 cells, or those treated with poly(ethylene glycol) alone in the absence of membranes served as control. As shown in Table III, only those cells that possessed the appropriate histocompatibility antigen, either naturally or by fusion, were killed by the immune lymphocytes.

As observed above for the function of the fused thyrotropin receptor in the recipient cells, cells containing the fused histocompatibility antigens could be recognised by the sensitised killer cells only within 60–100 min following fusion. No measurable killing was observed if the fused cells were incubated with the killer cells at, or after, approx. 150 min following fusion. This observation suggested that the fused membrane proteins may be destroyed or internalised by the recipient cells over a period of time.

Fusion of FITC-labeled membrane proteins into cells. Fluorescence microscopy of the fused membrane components

In order to examine the physical characteristics and fate of the fused membrane proteins, FITC-labeled membranes from human thyroid or EL4 cells were fused into the recipient cells. Fluorescence photomicroscopy showed that the fused membranes were incorporated into the plasma membranes of the recipient cells, though their distribution was not always uniform (Fig. 1a). Some clusters of membranes were seen fused to recipient cells. These could not be removed by washing (Fig. 1b). Within approx. 30 min the fluorescence began to patch and internalise (Fig. 1c). This process was found to be accelerated by the addition of hormone or the appropriate antibody to cell surface receptors.

Measurement of the lateral diffusion of the fused membranes components

Lateral diffusion of membrane components was measured by the technique of fluorescence recovery after pattern photobleaching (FRAPP). When measurements were made at 23°C approx. 90% of the fused membranes were found to be immobile while the remaining 10% diffused slowly (approx. 10^{-10} cm^2/s).

Discussion

The experiments described in this communication show that the thyrotropin receptors can be transferred from human thyroid membranes and coupled to the adenylate cyclase of a foreign living cell. The receptor appears to remain functional in the membrane of the recipient cell for approx. 100 min. During the initial 15–25 min following fusion, approx. 10% of the fused membrane proteins

diffused very slowly (10^{-10} cm^2/s) while the remainder were less mobile (10^{-11} cm^2/s). They then patch and internalise making the recipient cell no longer responsive to stimulation (of adenylate cyclase) by thyrotropin or TSI. The enzyme, however, remains responsive to stimulation with NaF and Gpp[NH]p.

Patching and internalisation of the fused membranes takes place both in the absence and presence of the thyroid stimulators, though it is relatively fast in their presence. The time period for patching and internalisation varies from one recipient cell type to another. Recipient cells grown as adherent monolayers seemed to internalise the fused membrane proteins at a more rapid rate (about 90 min) than cells grown in suspension (about 150 min).

Parallel experiments with the fusion of specific membrane histocompatibility antigens showed a similar behaviour. Fusion of H-2^b (or H-2^d) membranes into cells of the H-2^k haplotype rendered them susceptible to cytolysis by lymphocytes sensitised to H-2^b (or H-2^d) for the initial 100 min following fusion. If the targets were presented after this time, they were not killed.

Selective internalisation of specific membrane proteins [13] and fused lipids [14] has been observed in other systems. Petty et al. [13] have observed that during specific antibody dependent phagocytosis of lipid vesicles by macrophages, Fc but not C3b receptors are depleted from the cell surface. It appears that the fused cell surface components (hormone receptors or histocompatibility antigens) become refractory to hormone mediated stimulation of adenylate cyclase or recognition by sensitised lymphocytes because they are destroyed or selectively internalised by the recipient cell.

Acknowledgements

This work was supported by grant No. 5R01 AI13587-07 from the National Institute of Health (H.M.McC.) and a gift from Mr. Brooks Walker.

References

1 Sutherland, E.W., Robinson, G.A. and Butcher, R.W. (1968) Circulation 37, 279–306
2 Cuatrecasas, P. (1974) Annu. Rev. Biochem. 43, 169–214
3 Bourne, H.R., Caffino, P. and Tomkins, G.M. (1975) Science 187, 750–752
4 Schramm, M. (1979) Proc. Natl. Acad. Sci. U.S.A. 76, 1174–1178
5 Poste, G. and Nicholson, G. (1980) Proc. Natl. Acad. Sci. U.S.A. 77, 399–403
6 Prujansky-Jacobovits, A., Volsky, D.J., Loyter, A. and Sharon, N. (1980) Proc. Natl. Acad. Sci. U.S.A. 77, 7247–7251
7 Maeda, T., Balakrishnan, K. and Mehdi, S.Q. (1983) Biochim. Biophys. Acta 731, 115–120
8 Rapoport, B. (1976) Endocrinology 98, 1189–1197
9 Harper, J.F. and Brooker, G. (1975) J. Cyclic Nucleotide Res. 1, 207–218
10 Smith, B.A. and McConnell, H. (1978) Proc. Natl. Acad. Sci. U.S.A. 75, 1759–2763
11 Smith, L.M., Parce, J.W., Smith, B.A. and McConnell, H.M. (1979) Proc. Natl. Acad. Sci. U.S.A. 76, 4177–7179
12 Smith, B.A., Clark, W.A. and McConnell, H.M. (1979) Proc. Natl. Acad. Sci. U.S.A. 76, 5641–5645
13 Petty, H.R., Hafeman, D.G. and McConnell, H.M. (1980) J. Immunol. 125, 2391–2396
14 Struck, D.K. and Pagano, R.E. (1980) J. Biol. Chem. 255, 5405–5410