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Lateral transport on cell membranes: Mobility of concanavalin A receptors on myoblasts

(receptor mobility/membrane diffusion/photobleaching recovery)

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ABSTRACT **We report measurements of the lateral mobility of fluorescent labeled concanavalin A receptor complexes on the plasma membrane of cultured myoblasts of rat. Transport rates were measured by observing the recovery of fluorescence in a small region of the cell surface initially photobleached irreversibly by an intense, focused laser light pulse. Under different conditions we measured effective diffusion coefficients of the receptor complexes in the range $8 \times 10^{-12} \leq D \leq 3 \times 10^{-11}$ cm²/sec which is two orders of magnitude lower than we found for a fluorescent lipid probe, $D \simeq (8 \pm 3) \times 10^{-9}$ cm²/sec. This large difference and the presence of apparently immobile concanavalin A receptors suggests that factors beyond the fluidity of the phospholipid bilayer membrane matrix control the rate of lateral transport of the complexes. Effective mobilities of the complexes decrease with increases in the valence, dose, and occupation time of the lectin on the membrane. These properties imply an aggregation of the lectin–receptor complexes. Mobilities are not influenced by azide, colchicine or preincubation at low temperature. Cytochalasin B and low temperatures, during the time of measurement, decrease the lateral transport rate.**

We have directly measured the rate of lateral transport of fluorescent labeled concanavalin A (Con A) receptors on the plasma membrane of cultured myoblasts of rat. This paper presents a brief description of our methods and a preliminary account of our observations.

The translational mobility of membrane proteins and their redistribution after binding antibodies or lectins have recently attracted much attention (1–9). These phenomena have been discussed in terms of the fluid mosaic model (10) according to which membrane proteins are embedded in a fluid-like lipid bilayer, so that their translational and rotational diffusion are limited mainly by the viscosity of the lipid matrix (1, 10). The first experimental demonstration of lateral transport of membrane proteins was reported by Frye and Edidin (5). They measured the intermixing times of transplantation antigens after heterokaryon fusion and deduced an apparent diffusion constant of $D \sim 10^{-10}$ cm²/sec. A wide range of apparent diffusion coefficients of membrane proteins has been obtained in other systems: 1 to 2×10^{-9} cm²/sec for transplantation antigens on a muscle fiber (7), 4 to 5×10^{-9} cm²/sec for rhodopsin in amphibian discs (8), and $<3 \times 10^{-12}$ cm²/sec for nonspecifically labeled proteins in erythrocyte membranes (9). The patching (11) and capping (2, 12) of antibodies and lectins on cell surfaces are visible manifestations of protein mobility in cell membranes. Metabolic inhibitors prevent capping (12) but not patching (11). Moreover, colchicine and colcemid, inhibitors of microtubule assembly (13, 14), affect capping in lymphocytes, and suggests that these internal structures are somehow involved in the translational mobility of some membrane components (15, 16).

It is therefore of great interest to directly observe the rate of lateral transport of membrane proteins and lipids in order to test the fluid mosaic model. We have chosen Con A for our first investigation of lateral transport in membranes because its effects on lateral and rotational mobility have been invoked to explain or characterize a number of interesting cellular phenomena (3, 17–19).

We have developed a versatile capability for measuring the lateral transport on cell membranes of fluorescence labeled objects with effective diffusion coefficient D from $\sim 10^{-12}$ to $\sim 10^{-5}$ cm²/sec. For $D \gtrsim 10^{-9}$ cm²/sec we use fluorescence correlation spectroscopy (FCS) (20). For lower mobilities, we have adopted photobleaching methods (8, 9) and have developed a "fluorescence photobleaching recovery" (FPR) method that is accurate and convenient for $D \lesssim 10^{-9}$, and like FCS capable of discriminating diffusion from uniform driven transport processes and mobile from immobile fluorophore populations.

MATERIALS AND METHODS

Succinyl Con A (S-Con A) was prepared and purified according to the method of Gunther *et al.* (21). Tetramethyl rhodamine isothiocyanate derivatives of Con A and S-Con A were synthesized according to the method of Goldman (22). The labeled Con A and S-Con A, at concentrations of 3 mg/ml and 5 mg/ml, respectively, were kept in phosphate-buffered saline solution containing 1 M NaCl at −20°. Cells were incubated with labeled lectins diluted to various concentrations in Hanks' balanced salt solution (BSS) (0.5–10 μg/ml). One milliliter of the diluted labeled lectin was added to a 35 mm petri dish containing the rat myoblasts. Anti-Con A was a Miles-Yeda product; colchicine was a gift of Dr. T. Podleski. Cytochalasin B was obtained from Imperial Chemical Industry; Hanks' BSS from Grand Island Biological Co.; methyl α-D-mannopyranoside (α-MeMan) from Sigma Chemical.

L-6 rat embryo myoblasts (obtained from E. Racker) (23) were grown in 35 mm petri dishes in Dulbecco's Modified Eagle's medium (Grand Island Biological Co.) containing 2.5% fetal calf serum, 2.5% calf serum, 20 mM *N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid (Hepes) buffer (Sigma) and 22 mM bicarbonate buffer, both at pH 7.4. Prior to labeling, the cells were washed twice with 1 ml of Hanks' BSS. For the FPR experiments the cells were incubated with labeled lectins for 5–15 min at 37°, then washed with 1 ml of Hanks' BSS three times. Some additional experiments using 3T3 mouse fibroblasts (obtained from E. Racker) or primary human embryo fibroblasts (obtained from E. Mayhew) yielded results similar to L-6

Abbreviations: Hanks' BSS, Hanks' balanced salt solution; Con A, concanavalin A; S-Con A, succinyl concanavalin A; FITC, fluorescein isothiocyanate; FPR, fluorescence photobleaching recovery; FCS, fluorescence correlation spectroscopy; diI, 3,3'-dioctadecylindocarbocyanine iodide; α-MeMan, methyl α-D-mannopyranoside.

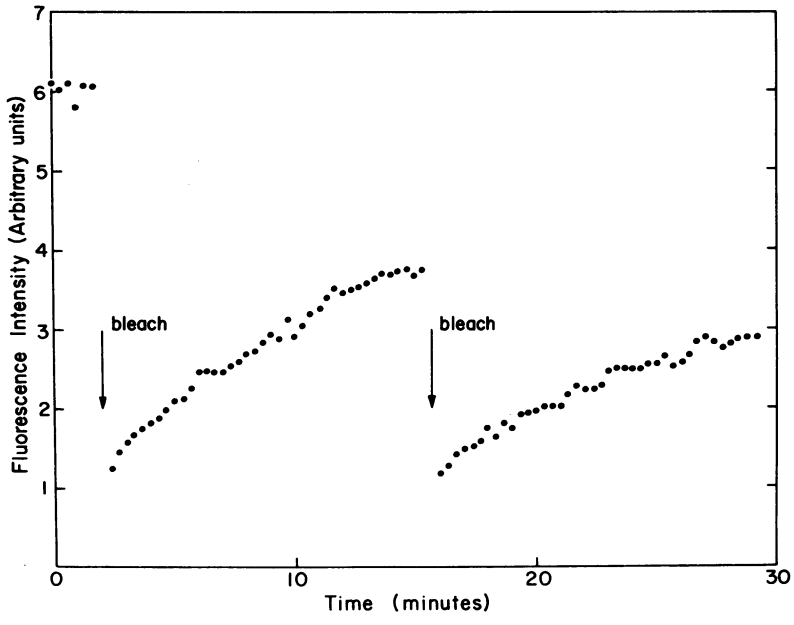

FIG. 1. A photobleaching recovery trace of fluorescent succinyl Con A (5 μg/ml) bound to a myoblast. This is a typical recovery curve with some wavering modulation of the recovery rate. Temperature, 23°. Fractional recoveries, defined as the total fluorescence recovered divided by the fluorescence change due to bleaching, are 55% after the first bleach, and 75% after the second.

cells. All FPR measurements were done on the original 35 mm petri dishes in which the cells were grown. The fluorescent lipid analogue 3,3′-dioctadecylindocarbocyanine iodide (dil; a gift from A. Waggoner) was incorporated into the plasma membrane by incubating the myoblasts with 1 ml of 3 μg/ml of dye in Hanks' BSS containing 1% (vol/vol) ethanol for 15 min at 37°.

The apparatus for the FPR measurements, constructed in our laboratory, was designed around a standard Zeiss Universal microscope. It will be described in detail elsewhere (24, 25, ‡). Light from a krypton laser at 520.8 nm is focused on a small area of about 1 μm radius on the surface of a labeled cell adhering to the petri dish. Exposure to 1 mW of laser power for 3 sec produces an initial gradient of fluorophore concentration. This exposure time is enough to photobleach about 70% of the initial fluorescence intensity and is short compared to the observed characteristic times for transport. The course of the fluorescence recovery is monitored with the laser intensity attenuated a thousandfold, and chopped to a duty cycle of 3 sec every 20 sec to minimize further bleaching. After the monitored fluorescence has reached a stable plateau, a second bleach is often performed under the same conditions. Some of the recovery curves show a wavering modulation (see Fig. 1) which may be attributed to membrane or cell movement relative to the beam. The nonwavering component of the photobleaching recovery data is usually analyzed simply in terms of $\tau_{1/2}$ which is defined as the time required for half of the observed fluorescence recovery to occur.§ [Note that diffusional mobility is proportional to D and inversely proportional to $\tau_{1/2}$.] Some experimental recovery curves were also compared to theoretical curves for transport by diffusion and for uniform driven motion. A detailed description of the derivation and experimental testing of these procedures will be published elsewhere (25, ¶).

RESULTS

1. The mobility and aggregation of Con A–receptor complex

We find that the mobility of the complexes depends on the valence and concentration of lectin with which the cells are incubated, and the time interval during which the lectin has occupied the cell surface. Fig. 1 presents typical data from an experiment with S-Con A. The recovered fluorescence intensity never reaches the initial value after the first bleach, while much higher fractional recovery is obtained after the second and subsequent bleaches. This result demonstrates that part of the lectin-binding sites are immobile on the time scale of the experiment.

Fig. 2 presents values of $\tau_{1/2}$ for tetravalent Con A and divalent S-Con A measured at various times after incubation with various concentrations of lectin (0.5–10 μg/ml). The figure clearly shows that the rate of lateral transport decreases with occupation time and decreases more rapidly the higher the Con A dose ($8 \times 10^{-12} \leq D \leq 3.3 \times 10^{-11}$ cm^2/sec). This strong inhibition of mobility is not seen with S-Con A, which is mobile over the entire range of doses and times studied, although it eventually moves somewhat more slowly at higher lectin doses (1.3×10^{-11} cm^2/sec $\leq D \leq 3.3 \times 10^{-11}$ cm^2/sec). These observations are consistent with earlier work (2, 3) which had previously shown that the Con-A receptor complexes aggregate on the cell surface. During our mobility measurements, many submicron patches were visible by fluorescence microscopy but there was no evidence that patch migration contributes to the mobility determining $\tau_{1/2}$. The values of $\tau_{1/2}$ were highly reproducible from cell to cell and within each individual cell, but the fractional recovery varied substantially within individual cells.

‡ D. Koppel, J. Schlessinger, D. Axelrod, E. L. Elson, and W. W. Webb, manuscript in preparation.

§ For diffusion in a plane the diffusion coefficient D equals $(w^2/4\tau_{1/2})\gamma$ where w is the $1/e^2$ radius of the focused, approximately gaussian beam, and γ accounts for the amount of bleaching and beam profile: $\gamma \cong 1.3$ in these experiments (‡). Note that the fluorescence may originate from the two closely spaced upper and lower surfaces of the cell.

¶ D. Axelrod, E. Koppel, J. Schlessinger, W. W. Webb, and E. L. Elson, manuscript in preparation.

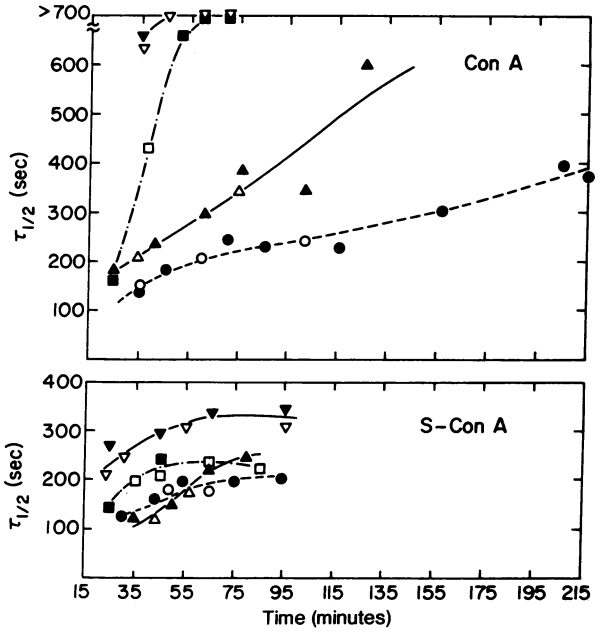

FIG. 2. The $\tau_{1/2}$ of photobleached fluorescent Con A (upper) and S-Con A (lower) bound to myoblasts at various doses and various time intervals after the incubation was started: 0.5 μg/ml (●); 2 μg/ml (▲); 5 μg/ml (■), and 10 μg/ml (▼). The open symbols represent experiments done under similar conditions but on myoblasts in another petri dish. Temperature, 23°. The amount of bound Con A depends on the dose. At a dose of 10 μg/ml with our incubation conditions, there are approximately 3×10^3 Con A molecules bound per square micron of cell surface.

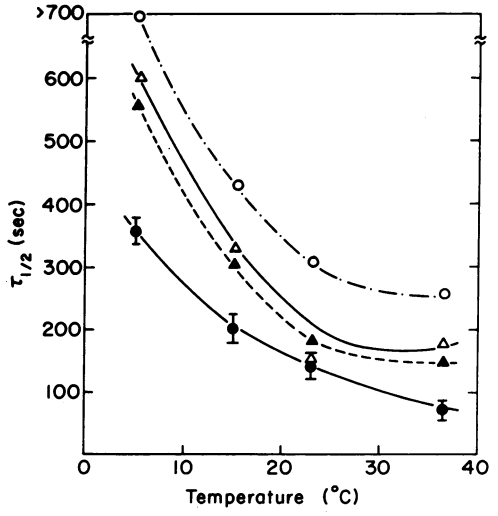

FIG. 3. The $\tau_{1/2}$ of photobleached fluorescent S-Con A and Con A (5 μg/ml) bound to myoblasts as a function of temperature. The $\tau_{1/2}$ for S-Con A represents an average of five measurements taken at varying times after labeling since at that dose the rate of mobility of S-Con A is only slightly time dependent. The error bars for the filled circles represent the standard deviation for each point measured for S-Con A. The mobility of Con A at this dose markedly depends on time. Each point represents a single experiment: 15 min after incubation ▲, 35 min △, and 50 min ○, respectively. The cells were preincubated for 15 min at the temperature at which mobility measurements were taken.

Direct evidence that aggregation can inhibit the mobility of the lectin–receptor complex was obtained by adding anti-Con A antibodies at a concentration of 100 μg/ml to cells prelabeled with either S-Con A or Con A. This treatment rapidly eliminates fluorescence recovery and thus abolishes lectin mobility. Because the antibody binds only to the lectin molecules and not to the cell surface, we conclude that this dramatic effect on lectin mobility results from the crosslinking of adjacent lectin molecules and that the mobile Con A molecules are bound at the exterior surface and not inside the cell.

That the observed recovery curves do measure the mobility of Con A which remains attached to the cell surface receptors, rather than a trivial effect like dissociation and reassociation of Con A, was confirmed by two direct experiments. First, the myoblasts were prefixed with 3% glutaraldehyde for 2 hr, washed for 30 min with Hanks' BSS, and then labeled with S-Con A or Con A. No mobility could be detected. Second, we tested the ability of α-MeMan, a specific competitive ligand for Con A binding sites, to displace Con A or S-Con A from the cell surface. Cells were incubated for 10 min with Con A or S-Con A at 5 μg/ml and then, after varying lengths of time, were exposed to one ml of 100 mM of α-MeMan for 10 min. Measurements of fluorescence intensities from 10 μm diameter areas of several cells indicated that both Con A and S-Con A were irreversibly bound to the cell surface after ~20 min, the occupation time for our earliest mobility measurements.

2. Pharmacological and temperature effects

Further insight into the mechanism of receptor transport was obtained by treating myoblasts with substances which affect particular cellular activities or structures. The metabolic inhibitor sodium azide at 10 mM does not significantly affect $\tau_{1/2}$ for either Con A or S-Con A. It does, however, reversibly reduce the wavering character of the recovery curves. Incubation of the myoblasts for 45 min with colchicine (1–100 μM) at 37°, with subsequent removal of the drug and labeling with lectin, influenced neither the $\tau_{1/2}$ nor the wavering modulation. FPR measurements at 23° were similarly unaffected by preincubation of the myoblasts for 30 min at 4° and exposure to lectin for a further 15 min at 4°. Low temperature and colchicine are expected to inhibit microtubule assembly (15). In contrast, preincubation with cytochalasin B at 10 μg/ml for 30 min prior to labeling with lectin (at 5 μg/ml) clearly does affect lectin mobility. After 30 min of lectin occupancy, $\tau_{1/2}$ of S-Con A receptor complexes is 550 ± 85 sec while $\tau_{1/2}$ for Con A is 600 ± 85 sec. A separate measurement of transport of dil in cells treated with cytochalasin B demonstrated that this drug had no effect on the viscosity of the lipid membrane matrix.

Con A and S-Con A mobilities are highly dependent on the temperature of the cells during the FPR measurements (see Fig. 3). At low temperatures there is slow, unwavering fluorescence recovery. Increasing the temperature causes an increase of the mobilities and the amplitude of the wavering modulation. The fraction of fluorescence recovery in cells, exposed to poisons and high or low temperatures, was within the range of values observed in untreated cells.

3. The diffusion of a fluorescent lipid probe

The diffusion coefficient of the lipid probe, dil, incorporated into the cell membrane, was measured by FCS to be $D = (8 \pm 3) \times 10^{-9}$ cm²/sec. This value is consistent with the lower limit $D > 10^{-9}$ cm²/sec obtained by FPR at 23° with virtually complete fluorescence recovery. It increased with temperature roughly in proportion to the diffusion coefficient of receptor complexes. These results clearly indicate that all of the dil is free to move in a continuous phospholipid matrix on a scale substantially greater than a micron.

Table 1. Mobilities of various components of the myoblast membrane

Probe	Labeled component	Method	Apparent diffusion coefficient (cm^2/sec)	Mobile fraction
S-Con A (15 min after cell labeling with 2 $\mu g/ml$)	Con A receptors	FPR	$(4.2 \pm 0.8) \times 10^{-11}$	$\sim 2/3$
Con A (15 min after cell labeling with 2 $\mu g/ml$)	Con A receptors	FPR	$(2.9 \pm 0.8) \times 10^{-11}$	$\sim 2/3$
diI	Phospholipid matrix	FPR	$> 10^{-9}$	~ 1
		FCS	$(8 \pm 3) \times 10^{-9}$	—
FITC[a]	Membrane proteins labeled with FITC	FPR	$(2.2 \pm 1.0) \times 10^{-10}$	$\sim 1/4$

[a] Cells were incubated for 10 min with 1 ml FITC (at concentrations from 25 to 200 $\mu g/ml$) at pH 7.6 . The values are shown ±SD.

DISCUSSION

Our results depart in two fundamental respects from expectations based on the fluid mosaic model. First, we have detected a class of complexes that display no lateral motion on the time scale of our experiments. This is indicated by the consistent failure of the fluorescence to regain its initial value, especially after the first bleach. A preliminary experiment in which membrane proteins were labeled with FITC shows an even larger fraction of "immobile" proteins (see Table 1). Second, the effective diffusion coefficient of the mobile lectin–receptor complexes and FITC-labeled proteins were substantially lower than is predicted for individual complexes in a fluid membrane with viscosity between one and ten poise or than was seen for rhodopsin in retinal disc membranes (8).

One possible explanation is that the lipid matrix of myoblast membranes is more viscous than those of systems previously studied. Preliminary measurements of the diffusion coefficient of an amphipathic lipid analogue in myoblast membranes, however, yielded a value consistent with those earlier studies (see Table 1).

Another possible explanation of the low lateral mobility of the lectin-receptor complexes is that they form aggregates which may be transported more slowly than individual complexes perhaps due to increased viscous drag or for some other reason. The decrease of mobility on increasing dose, valence, and time of occupancy of the lectin imply aggregation of Con A–receptor complexes on the membrane. Furthermore, cross-linking by anti-Con A antibodies dramatically reduces the mobility of the complexes. Two additional experiments suggest, however, that lectin-induced aggregation of the complexes is insufficient to account entirely for the slow receptor transport. First, surface proteins labeled with FITC show significantly reduced fractional recovery, and transport rates which, though 2- to 5-fold faster than the fastest lectin–receptor complexes, are nevertheless almost two orders of magnitude slower than diI diffusion (26, ‖, and Table 1). Since labeling of proteins by fluorescein is not expected to enhance aggregation, the slow transport of some proteins and the immobility of others seems most likely to be a preexisting condition. Second, transport rates measured soon after lectin binding (at ~20 min for several lectin doses and at 10 min at a dose of 5 $\mu g/ml$) do not show the dependence on lectin valence and dose attributed to lectin-induced aggregation (see Fig. 2). Moreover patches visible by fluorescence microscopy appeared only after 20 min when mobilities of Con A and S-Con A began to differ. Hence aggregation of complexes due to Con A seems to have begun only after 20 min.

The presence of patches during our mobility measurements indicates some form of aggregation. However, the homogeneity and reproducibility from area to area of the measurements of $\tau_{1/2}$ tends to exclude involvement of patches in the unwavering component of fluorescent recovery.

The experiments with pharmacological agents probe physiological aspects of the mechanism of receptor transport. Treatment with azide does not alter the mobility of the receptors but does reduce the wavering modulation. Cytochalasin B reduces the rate of transport but does not affect the wavering modulation. The temperature of the cells during the experiments affects both the mobility and the wavering modulation, while both colchicine and preincubation at 4° affect neither. That the effective mobility and the wavering modulation of transport are affected in different ways implies that these two phenomena are controlled by different mechanisms.

It is difficult to interpret the temperature dependence since both diffusion and active processes are expected to be temperature dependent. Cytochalasin B affects the structure of some (16) but perhaps not all (27) microfilaments and abolishes cell movement (16). The effect of cytochalasin B which we observed tentatively suggests that microfilaments are involved in receptor mobility or its inhibition.

In addition to the physiological experiments described above, we have also pursued physical characterization of the mechanism of transport. We have derived the expected shape of fluorescence recovery curves for diffusion, constant velocity flow, and for combinations of the two.¶ Although the presumed heterogeneity of the structure of Con A receptors (28) and therefore of their transport introduces problems for this approach, preliminary analysis shows that the functional form of the measured recovery curves conforms better with the theoretical curve for diffusion than that for uniform flow.

In conclusion the most striking results of this study include the following: (*i*) Con-A receptor complexes and FITC-labeled protein complexes divide into two classes. One appears immobile in our experiments; the other moves with an effective diffusion coefficient in the range $8 \times 10^{-12} \leq D \leq 1.6 \times 10^{-10}$ cm^2/sec. This is two orders of magnitude slower than the diffusion of a fluorescent lipid probe incorporated in the plasma membrane. (*ii*) The mobility of Con A–receptor complexes depends on the valence, dose and time of occupancy of the lectin on the receptor in a way that implies surface aggregation

‖ J. Schlessinger, D. Axelrod, D. Koppel, W. W. Webb, and E. L. Elson, manuscript in preparation.

of the Con A–receptor complexes. (*iii*) Treatment with azide, colchicine, or low temperature preincubation does not detectably influence the effective rates of lateral transport of Con A–receptor complexes suggesting that oxidative phosphorylation and microtubules are not strongly involved in the observed transport processes. (*iv*) The rate of transport is reduced by cytochalasin B and by maintaining cells at low temperature during the fluorescence recovery measurements. The cytochalasin B effect suggests involvement of microfilaments in the mechanism of receptor transport.

Although we have not yet determined the mechanisms of macroscopic lateral transport of the Con A receptor, these experiments begin a promising phenomenological characterization of the processes. They indicate the utility of the fluorescence photobleaching recovery method. We expect that further work along these lines on receptors of higher specificity will lead to a clearer picture of the mechanisms of transport processes on cell surfaces.

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1. Edidin, M. (1974) *Annu. Rev. Biophys. Bioeng.* **3**, 179–201.
2. Taylor, R. B., Duffus, W. P. H., Raff, M. C. & DePetris, S. (1971) *Nature New Biol.* **233**, 225–229.
3. Yahara, I. & Edelman, G. M. (1972) *Proc. Natl. Acad. Sci. USA* **69**, 608–612.
4. Cherry, R. J. (1975) *FEBS Lett.* **55**, 1–7.
5. Frye, L. D. & Edidin, M. (1970) *J. Cell Sci.* **7**, 319–335.
6. Petit, V. A. & Edidin, M. (1974) *Science* **184**, 1183–1184.
7. Edidin, M. & Fambrough, D. (1973) *J. Cell Biol.* **57**, 27–37.
8. Poo, M.-m. & Cone, R. A. (1974) *Nature* **247**, 438–441.
9. Peters, R., Peters, J., Tews, K. H. & Bähr, W. (1974) *Biochim. Biophys. Acta* **367**, 282–294.
10. Singer, S. J. & Nicolson, G. L. (1972) *Science* **175**, 720–731.
11. DePetris, S. & Raff, M. C. (1973) *Nature New Biol.* **241**, 247–259.
12. DePetris, S. & Raff, M. C. (1972) *Eur. J. Immunol.* **2**, 523–535.
13. Weber, K., Pollack, R. & Bibring, T. (1975) *Proc. Natl. Acad. Sci. USA* **72**, 459–463.
14. Goldman, R. D. & Knipe, D. M. (1972) *Cold Spring Harbor Symp. Quant. Biol.* **37**, 523–534.
15. Edelman, G. M. (1974) in *Control of Proliferation in Animal Cells*, eds. Clarkson, B. & Baserga, R. (Cold Spring Harbor Laboratories, Cold Spring Harbor, N.Y.), pp. 357–377.
16. Bretscher, M. S. & Raff, M. C. (1975) *Nature* **258**, 43–49.
17. Inbar, M. & Sachs, L. (1969) *Proc. Natl. Acad. Sci. USA* **63**, 1418–1425.
18. Burger, M. M. & Martin, G. C. (1972) *Nature New Biol.* **237**, 9–12.
19. Douglas, S. D., Kamin, R. M. & Fudenberg, H. H. (1969) *J. Immunol.* **103**, 1185–1195.
20. Elson, E. L. & Webb, W. W. (1975) *Annu. Rev. Biophys. Bioeng.* **4**, 311–334 (and references cited therein).
21. Gunther, G., Wang, J., Yahara, I., Cunningham, B. & Edelman, G. (1973) *Proc. Natl. Acad. Sci. USA* **70**, 1012–1016.
22. Goldman, M. (1968) *Fluorescent Antibody Methods* (Academic Press, New York and London).
23. Yaffe, D. (1968) *Proc. Natl. Acad. Sci. USA* **61**, 477–483.
24. Koppel, D., Axelrod, D., Schlessinger, J., Elson, E. L. & Webb, W. W. (1976) *Abstr. Biophys. Soc.* (Seattle).
25. Axelrod, D., Koppel, D., Schlessinger, J., Elson, E. L. & Webb, W. W. (1976) *Abstr. Biophys. Soc.* (Seattle).
26. Edidin, M., Zagyansky, Y. & Lardner, T. J. (1976) *Science* **191**, 466–468.
27. Wessells, N. K., Spooner, B. S. & Luduena, M. A. (1973) in *Locomotion of Tissue Cells* (Ciba Foundation Symposium 14, new series) (Elsevier Excerpta Medica, North Holland, Amsterdam), pp. 53–76.
28. Sela, B. A., Wang, J. L. & Edelman, G. M. (1975) *J. Biol. Chem.* **250**, 7535–7538.