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Analysis of Cell Surface Interactions by Measurements of Lateral Mobility

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Interactions of cell surface components with one another and with structures inside and outside the cell may have important physiological functions in the transmission of signals and the assembly of specialized structures. These interactions may be detected and analyzed through their effects on the lateral mobility of cell surface molecules. Measurements by a fluorescence photobleaching method have shown that in general lipid-like molecules diffuse rapidly and freely through the plasma membrane, whereas proteins move much more slowly or appear to be immobile. This dichotomy has been supposed to result from forces beyond the viscosity of the lipid bilayer, which specifically retard the diffusion of membrane proteins. This general picture should be qualified, however, by noting that the lateral mobility of lipid-like molecules can be influenced in detail by changes in the state of the plasma membrane such as result from mitosis or fertilization. The interactions of cell surface proteins that limit their lateral mobility are unknown. The effects of binding concanavalin A to localized regions of cell surface show that these interactions can vary in subtle and complex ways. It may soon be useful to interpret mobility experiments in terms of simple reaction models that attempt to describe surface interactions in physicochemical terms. More experimental data are needed to carry out this program and to relate interactions that affect mobility to the structural connections between cell surface components and the cytoskeleton, which have been detected by biochemical methods and electron and immunofluorescence microscopy.

Key words: fluorescence photobleaching, cell surface, cytoskeleton, lateral mobility, membrane interactions

INTRODUCTION

Important physiological functions that occur at the surface of an animal cell depend on dynamic interactions of molecules embedded in the plasma membrane with one another and with molecules and structures inside and outside the cell. For example, it has recently been recognized that activation of specific processes in various kinds of cells by polypeptide hormones such as insulin and epidermal growth factor (EGF) [1, 2] and on mast cells and

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basophils by immunoglobulin E [3] may involve the rapid association in the plasma membrane of ligand receptor complexes. Apparently a limited aggregation of these complexes is needed to transmit into the cells the signals required to trigger their specific responses. It also now appears that formation of (perhaps somewhat larger) aggregates is required for clearing ligand–receptor complexes from the plasma membrane by a specific endocytic pathway that involves coated vesicles [4].

Similarly, the formation of specialized structures at the cell surface must require interactions among plasma membrane molecules or with cytoplasmic components. These specialized structures are involved in various cellular functions and include, for example, subsynaptic concentrations of acetylcholine receptor, an intrinsic membrane protein that mediates neuromuscular signal transmission [5]; fibers of the extrinsic glycoprotein, fibronectin, which contributes to the formation of a pericellular connective matrix [6]; and coated regions of the plasma membrane, which may contain receptors of various kinds as well as the protein clathrin [7] and which mediate the specific endocytosis of a wide range of ligand–receptor complexes, including those of insulin, EGF, low-density lipoprotein, and α_2-macroglobulin [8–11].

Characterization of the specific interactions of cell surface components and of the pathways by which specialized surface structures are assembled present important problems for cell physiology. Recently, methods have been developed that allow a physicochemical approach to these problems. This paper discusses the use of measurements of macroscopic lateral mobility to gain mechanistic information about cell surface interactions and assembly processes. This approach is based simply on the idea that the lateral mobility of a molecule in the plasma membrane will be retarded to the extent that it interacts with other surface or cytoplasmic molecules or supramolecular structures. As we shall see, the nature and extent of this retardation depends both on the mobility of the interacting structures and on the strength and kinetics of the interactions. Therefore, in favorable circumstances it may be possible to obtain information about these several properties from mobility measurements.

APPROACH AND METHOD

The lateral and rotational mobility of cell surface molecules can be measured in different ways using methods based on fluorescence [12], magnetic resonance [13], or other properties [14]. We use a fluorescence photobleaching recovery (FPR) method that is relatively simple, quantitative, and readily applicable to individual cells living in culture without apparent serious perturbation of cellular properties [15]. A cell surface component is labeled with a fluorescent tag. The fluorescence from a small open region of the cell surface is measured using a microscope equipped with a sensitive photomultiplier tube [16]. The measured fluroescence (corrected for background) is proportional to the number of labeled molecules in the surface observation region. A brief intense pulse of light is used to "destroy" by irreversible photolysis a fraction of the labeled molecules in the region. Thus the fluorescence in the region is momentarily decreased below its initial level by an amount that depends on the intensity and duration of the bleaching pulse as well as the photochemical properties of the fluorophore. In the absence of further bleaching the measured fluorescence will recover due to transport of unbleached fluorophores from adjacent regions of the surface into the observed region. Hence, from this measured rate of fluorescence recovery, the rate of transport may be deduced [17]. Recovery may occur after diffusion or systematic flow of labeled molecules. Up to now almost all measurements that have been made are accounted for simply in terms of diffusion.

As a simple model for an interaction involving a mobile surface component, we may consider the reaction

$$A + B \underset{k_b}{\overset{k_f}{\rightleftharpoons}} C$$

We shall suppose that A is a nonfluorescent molecule or structure, which because of its size or its connection with other cellular components, is either immobile or slowly moving with diffusion coefficient D_A. The membrane component B is fluorescent and and, when free, moves with a diffusion coefficient, D_B. The effective mobility of B is, however, retarded by its interaction with A to form the complex C. For simplicity we shall suppose that interaction with B does not change the low mobility of A. Hence $D_A = D_C << D_B$. The slower the rate of diffusion of C and the stronger the interaction of A and B, the greater will be the retardation of B. Hence, the retardation depends on D_C and on the equilibrium constant $K = k_f/k_b = \bar{C}_C/\bar{C}_A\bar{C}_B$, where $\bar{C}_X$ is the equilibrium concentration of X.

The kinetics of the interaction may be reflected in the experimental measurements in two different ways. If there is a substantial change in the fluorescence excitation or emission of B due to its interaction with A, then the chemical relaxation can be observed directly. This type of effect has been demonstrated in a study of the interaction of ethidium bromide with DNA by a closely related fluorescence fluctuation method [18]. The kinetics of reaction (1) may also be revealed by the nature of the retarding effect on the mobility of B. This will depend on the relative magnitudes of the characteristic times for fluorescence recovery by simple diffusion of B and by chemical relaxation. In an FPR experiment the characteristic time for diffusion of B, τ_B, is given as

$$\tau_B = w^2/4D_B$$

In this equation w is the radius of the observation region on the cell surface [17]. The characteristic time for chemical relaxation is given, assuming linearized chemical kinetics, by the well-known formula:

$$\tau_{chem} = R^{-1} = [k_f(\bar{C}_A + \bar{C}_B) + k_b]^{-1}$$

If the chemical reaction is slow compared to diffusional recovery of B – ie, $\tau_{chem} >> \tau_B$, – a given B molecule will be observed, for the most part, to be either free or complexed with A, but only rarely will it experience both states during the measurement interval. Therefore, if $D_B >> D_A$, the B molecules will appear to reside in two distinct mobility classes: fast, due to diffusion of free B with diffusion coefficient D_B, and slow, due to diffusion of C with diffusion coefficient D_C. For D_C sufficiently small, there may be negligible recovery of C during the measurement period, so that B molecules involved in the complex will appear to be effectively immobile. If, however, the chemical reaction is fast compared to the diffusional recovery of B – ie, $\tau_{chem} << \tau_B$, – each B molecule will react many times with A during the observed fluorescence recovery time. Then all B molecules will be retarded to a comparable extent, so that the FPR experiment will reveal a single mobility class with effective diffusion coefficient D_e [19]:

$$D_e = D_C f_C + D_B f_B$$

where

$$f_C = \frac{\bar{C}_C}{\bar{C}_B+\bar{C}_C} = \frac{K\bar{C}_A}{1+K\bar{C}_A} \quad \text{and} \quad f_B = 1 - f_C = \frac{\bar{C}_B}{\bar{C}_B+\bar{C}_B} = \frac{1}{1+K\bar{C}_A}$$

Hence, under these conditions, K may be determined by measurements of the effective diffusion coefficient of B at various values of $\bar{C}_A$. This principle has been applied by Borejdo to study the interaction of actin and myosin using a closely related fluorescence fluctuation approach [20]. A detailed theoretical analysis of the measurement of the kinetics of reaction [1] by this fluorescence fluctuation approach has been presented [21]. The analysis of the measurement of this system by FPR is fundamentally similar, although important differences in detail must be taken into account [19].

Since the characteristic time for diffusional recovery, τ_B (but not τ_{chem}), depends on the dimension of the observation region, the time scale of the experiment may be changed by varying w. This raises the possibility of probing the kinetics of a cell surface reaction by increasing w to pass from a condition in which two mobility classes are observed ($\tau_{chem} >> >> \tau_B$) to a condition in which B seems to move at a single "effective" rate ($\tau_{chem} << \tau_B$). Up to now this principle does not appear to have been experimentally applied. Nevertheless, experimental results are now available that indicate the presence of some interactions that are fast and others that are slow under conditions typically used for FPR measurements of surface mobility on animal cells (time scale ~ 0.1 sec to ~ 100 sec).

It is important also to ask to what extent the mobility measurements might be influenced by technical problems with the FPR method. Recently, there have appeared reports of photo-induced cross-linking of cell surface proteins by irradiation of fluorescein bound to surface molecules [22, 23]. In principle, this type of cross-linking could reduce the mobility of, and possibly even immobilize, cell surface proteins. Unfortunately, the conditions under which these experiments must be carried out in order to obtain sufficient material for biochemical analysis (relatively low intensities of excitation and long periods of exposure) are not comparable to those of FPR measurements (higher intensities, shorter exposure). Moreover, it appears that increasing intensity and decreasing the duration of exposure at constant total excitation energy decreases the amount of cross-linking [23]. Yet mobility measurements carried out using different excitation intensities and lengths of exposure show no effect of these variables on the measured mobilities [24]. This and other evidence [24, 25] leads us to conclude, at least provisionally, that photo-induced cross-linking of surface components does not strongly perturb the dynamic properties measured by FPR.

Cell Surface Lipids and Proteins

Different kinds of interactions seem to govern the rates of diffusion of cell surface lipids and proteins. Lipid-like molecules are typically observed to diffuse rapidly, and apparently homogeneously, in the plasma membranes of cultured cells. The diffusion coefficient of the lipid probe 3,3′-dioctadecylindocarbocyanine ("dil") is approximately 10^{-8} cm^2/sec in a number of different kinds of cells [26–28]. Usually the dil fluorescence recovers after a photo-bleaching pulse to nearly its initial prebleach level, indicating that most of the observed fluorescence is in a freely mobile form. In contrast, cell surface proteins appear to exist in both mobile and immobile forms, with the mobile molecules moving substantially more slowly than dil. Both nonspecifically labeled cell surface "proteins" [26] and several different surface receptors, including those that bind immunoglobulin E on mast cells [27], α-bungarotoxin on myotubes (acetyl-

choline receptor) [29], and insulin and epidermal growth factor on fibroblasts [30], have diffusion coefficients less than 10^{-9} cm^2/sec. Moreover, incomplete recovery of the fluorescence to its prebleach level indicates that some portion of each of these membrane components is immobile on the time scale of the measurement.

These results and other work with which they are consistent [14, 31] indicate that the mobility of the lipid probel dil is determined by the viscosity of the lipid bilayer matrix in which it is embedded. It would be simplest to suppose that the mobility of cell surface proteins is also limited only by the viscosity of this matrix. In fact, this does appear to be a reasonable view of the behavior of rhodopsin in amphibian rod outer segment disk membranes [32, 33]. The experimental results just described, however, argue on two counts against applying this viewpoint to typical animal cell surface membranes. First, a fraction of the proteins in these membranes is constrained, even to the extent that the proteins appear immobile in our measurements. Second, even the mobile proteins move far more slowly than the lipid probe and more slowly than would be expected simply from the greater viscious resistance due to the greater size of the protein molecules [33]. It has therefore been argued that interactions and forces in addition to the viscous resistance of the lipid bilayer must be restraining the mobility of membrane proteins [34]. The structural basis of these restraining interactions is entirely unknown. Recently, biochemical, electron microscopic, and immunofluorescence evidence of links between the cell surface and the cytoskeleton has been presented [35–40]. The effect of these links on mobility has yet to be demonstrated, however, Attempts to use agents such as colchicine and cytochalasin B, which disrupt (respectively) microtubules and microfilaments, have not led to definitive conclusions about the role of these cytoskeletal elements in determining the mobilities of cell surface components [26, 34]. Similarly, little can yet be said about the dynamic characteristics of these interactions. From the viewpoint of the simple reaction model described above, cell surface proteins would seem to experience both slow and fast interactions to account, respectively, for the apparent immobilization of a fraction and the slow diffusion of the balance of these molecules.

A detailed analysis of the dynamics of well-characterized classes of cell surface proteins has yet to be performed. This should include measurements of the diffusion of membrane proteins reconstituted into model membrane systems to establish a baseline condition for the mobility of those molecules in the absence of restraints by cytostructural components [41–45]. Experiments of this kind must contend with questions of the fidelity of the reconstituted system to the natural disposition of the membrane protein. It could also be valuable to attempt a more detailed analysis on cell surfaces of the range of mobilities experienced by specific proteins and of the degree of variation of mobility at different positions on the cell. This kind of analysis is complicated by systematic and random errors, which are difficult to eliminate from measurements on living cells.

Modulation of Lipid and Protein Mobilities

The contrast between cell surface lipids, which more freely and rapidly in the plasma membrane, and proteins, which are constrained by unknown forces to much slower rates of diffusion, is consistent in measurements up to now [12, 34]. Nevertheless, interesting examples of modulation of lipid mobility by changes in the physiological condition of a cell or by interaction with a specific membrane component have recently been discovered. Edidin and Johnson [46] have shown that both dil and surface antigens on mouse ova become less mobile upon fertilization. The mechanism of this effect is still unknown. Edidin

and Johnson speculate, however, that the immobilization of cell surface components may be needed for several processes involved in embryonic development [46]. Studies on neuroblastoma cells have shown that the mobilities both of lipid probes and of surface antigens change in a systematic way over a two- to threefold range during the cell cycle [47]. The diffusion coefficients of both kinds of molecules are at a minimum during mitosis and increase during G_1. Then the diffusion coefficients of the lipid probes remain constant during S and G_2, before diminishing again during M. In contrast, the diffusion coefficients of surface antigens decrease gradually during S and G_2, with a small further reduction occurring during M. The mobilities of two structurally distinct lipid probes change similarly through the cell cycle. Therefore it seems likely that this results from a general change in membrane fluidity rather than from specific interactions with cellular structures. Moreover, the differences in behavior of the lipid probes and the membrane antigens indicate that factors in addition to lipid fluidity must influence the dynamic properties of these molecules (presumed to be mainly cell surface proteins).

Examples have recently have been found in which the mobilities of lipid probes are diminished by apparently specific interactions with identified membrane components. These were discovered in measurements of the diffusion of fluorescein-labeled ganglioside and ceramide analogs in the plasma membranes of cells infected by vesicular stomatitis and sindbis viruses [48]. Viral glycoprotein at the surface of infected cells may spontaneously form patches or can be induced to do so with bivalent antibody directed against the viral glycoproteins. Double-label fluorescence microscopic observations revealed that the fluorescein-labeled lipid probes were selectively concentrated into regions rich in viral glycoprotein. Measurements of the mobilities of the analogs in regions of high and low viral glycoprotein concentration indicated a complex pattern of behavior with decreases both in diffusion coefficients and in the fraction of mobile molecules. The mobility of dil, however, was the same in regions of high and low viral glycoprotein concentration, suggesting that the effects on the mobility of the analogs resulted from specific interactions rather than a more general effect on local membrane fluidity.

These results show that, although lipid-like molecules typically do move more freely than do proteins through the plasma membrane, nevertheless, even lipid probes may experience various kinds of interactions that influence their mobilities in detail.

The forces in addition to membrane viscosity that retard the motion of typical cell surface proteins may result from interactions with other cell surface components or with structures inside or outside the cell. In the plane of the membrane short-lived, possibly nonspecific, interactions can uniformly reduce the mobility of cell surface proteins without immobilizing them [44]. Long-lived specific interactions due, for example, to a cross-linking antibody [27] or to the binding of insulin or ECF to their receptors [30], can induce the formation of large immobile aggregates. Whether the immobility of these aggregates results from increased viscous resistance due to their larger size or from the enhancement of interactions with slow-moving or stationary structures is still uncertain [34].

Little is known yet about the effects on mobility of contact between the plasma membrane and the extracellular connective matrix. It has been shown that fibronectin fibers do not impede the diffusion of lipid probes and cell surface antigens [28]. Concanavalin A, however, is immobilized by binding directly to immobile fibronectin fibers [28].

Structural and biochemical evidence implicate interactions between cell surface components and the cytoskeleton both in immobilization [39] and in dynamic redistribution – eg, cap formation [37, 49, 50]. It is typically supposed that stable linkages

with the cytoskeleton are established in response to cross-linking surface proteins [39, 36, 49]. Even in the absence of an externally applied cross-linking agent, however, there may be interactions between the cytoskeleton and membrane proteins that are stable enough to survive gentle extraction by Triton X-100 [51]. It is reasonable to suppose that the linkages revealed in these studies must also influence cell surface mobility and that measurements of the lateral diffusion of membrane proteins should help to characterize the kinds of interactions involved. For example, it might be supposed that the immobilized fraction of labeled cell surface proteins indicated by incomplete recovery of fluorescence after photo-bleaching is due to the formation of stable cytoskeletal links. It is perhaps surprising, therefore, that the effects of anti-cytoskeletal agents such as colchicine and cytocholasin B have been so unrevealing [34].

An example of the potential complexity and subtlety of interactions affecting surface mobility has been provided by a study of the influence of concanavalin A (conA) on the lateral diffusion of plasma membrane antigens [52]. The conA was confined to localized regions of 3T3 cell surfaces by having first been reacted with platelets. Then the conA-platelets were bound in clumps to circumscribed regions of the cell surface. FPR measurements at various distances from the platelet clumps showed no effect on lateral diffusion until some 4% of the dorsal cell surface was covered by platelets. Beyond that point the diffusion coefficient of the antigens was reduced sixfold and remained at this reduced value at higher fractions of surface coverage by the conA-platelets. There was no effect observed on the fraction of *immobile* antigens as measured by the extent of fluorescence recovered, near 50% on average under all conditions tested. The extent of inhibition of mobility was not correlated with distance from the platelet clumps. When the experiment was repeated in the presence of anti-microtubule agents, a similar pattern of behavior was observed, except that antigen mobility was reduced three- rather than sixfold.

These measurements indicate that localized perturbations of the state of conA-receptors can inhibit mobility over the entire cell surface. The threshold and plateau of the inhibition suggest that the responsible interactions are initiated in a highly cooperative process. This process could be a polymerization or assembly of, for example, a cytoskeletal component, or it could be the activation of a diffusable enzyme; either could propagate the effect over the entire cell surface [52, 34].

It is interesting to consider the effects of local conA binding from the viewpoint of the simple reaction model, equation (1). It is simplest to suppose that the lack of effect on the fraction of recovered fluorescence, and therefore of immobile antigens, is due to failure of conA to influence long-lived interactions that constrain these molecules. (This analysis is complicated by the heterogeneous specificities of the anti-cell surface antibodies used to detect surface mobility in these experiments [52]. It is also possible that some sets of antigens were immobilized by short-lived interactions for which the binding equilibrium lay far to the right; $f_C \sim 1$, $f_B \sim 0$. Detected in isolation these molecules would appear to be entirely immobile rather than divided into mobile and immobile fractions. It is therefore important to determine the effect of local conA binding on the mobility of well-defined, homogeneous surface components.) The model further suggests that the sixfold reduction in the diffusion coefficients of the mobile antigens (B) could result from enhancement of interactions with anchorage components (A), which are rapid compared to the typical fluorescence recovery time (10–500 sec in these experiments). This enhancement could result from increasing the concentration of A available for reaction with B or by increasing the equilibrium constant K.

The threefold inhibition of mobility with characteristic threshold and plateau induced in the presence of anti-microtubule agents suggests that microtubules are not directly involved in the interactions of cell surface antigens that respond to localized conA binding. These interactions seem to be enhanced similarly in the presence and absence of the anti-microtubule agents. The twofold decreases in the extent of immobilization in the presence of these agents indicates that microtubules do have some role – perhaps in further stabilizing the surface-modulating assembly [49].

CONCLUSIONS

Measurements of lateral mobility indicate that cell surface components experience both transient and long-lived interactions. These interactions may modulate the kinetics of dynamic physiological processes such as the aggregation of hormone- and immunoglobulin-receptor complexes [1–4]. They may also be involved in the assembly of specialized cell surface structures [29]. It should eventually be possible to extend the characterization of these interactions by interpreting experimental measurements in terms of reaction models, of which equation (1) embodies an especially simple example. That model, for example, could be tested directly if the concentrations of components A and B could be systematically varied on the cell surface. Interpretation of interactions that limit mobility in structural and biochemical terms is a further challenge – one that will require the combined application of many experimental techniques. It is particularly interesting now to relate the interactions detected through their effects on mobility with those that have already been revealed by biochemical analysis and electron and immunofluorescence microscopy [35–40, 50, 51].

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REFERENCES

1. Kahn CR, Baird KL, Jarrett DB, Flier JS: Proc Natl Acad Sci USA 75:4209–4213, 1978.
2. Shechter Y, Hernaez L, Schlessinger J, Cuatrecasas P: Nature 278:835–838, 1979.
3. Segal D, Taurog J, Metzger H: Proc Natl Acad Sci USA 75:2993–2997, 1977.
4. Schlessinger J, Schechter Y, Willingham MC, Pastan I: Proc Natl Acad Sci USA 75:2659–2663, 1978.
5. Fertuck HC, Salpeter MM: Proc Natl Acad Sci USA 71:1376–1378, 1974.
6. Yamada KM, Olden K: Nature 275:179–184, 1978.
7. Pearse BMF: J Mol Biol 97:93–98, 1975.
8. Anderson RGW, Goldstein JL, Brown MS: Proc Natl Acad Sci USA 73:2434–2438, 1976.
9. Gordon P, Carpentier J-L, Cohen S, Orci L, Proc Natl Acad Sci USA 75:5025–5029, 1978.
10. Maxfield FR, Schlessinger J, Shechter Y, Pastan I, Willingham MC: Cell 14:805–810, 1978.
11. Brown MS, Goldstein JL: Proc Natl Acad Sci USA 76:3330–3337, 1979.

12. Schlessinger J, Elson EL: In Ehrenstein G, Lecar H (eds): "Biophysical Methods." New York: Academic Press (in press).
13. Chan SI, Bocian DF, Petersen NO: In Grell E (ed): "Membrane Spectroscopy." New York: Springer, Verlag (in press).
14. Edidin M: Ann Rev Biophys Bioeng 3:179–201, 1974.
15. Elson EL, Schlessinger J, Koppel DE, Axelrod D, Webb WW: In Marchesi VT (ed): "Membranes and Neoplasia: New Approaches and Strategies." New York: Alan R. Liss, 1976, pp 137–147.
16. Koppel DE, Axelrod D, Schlessinger J, Elson EL, Webb WW: Biophys J 16:1315–1329, 1976.
17. Axelrod D, Koppel DE, Schlessinger J, Elson E, Webb WW: Biophys J 16:1055–1069, 1976.
18. Magde D, Elson EL, Webb WW: Biopolymers 13:29–61, 1974.
19. Elson EL: in preparation.
20. Borejdo J: Biopolymers 18:2807–2820, 1979.
21. Elson EL, Magde D: Biopolymers 13:1–27, 1974.
22. Lepcock JR, Thompson JE, Kruuv J, Wallach DFH: Biochem Biophys Res Commun 85:344–350, 1978.
23. Sheetz MP, Koppel DE: Proc Natl Acad Sci USA 76:3314–3317, 1979.
24. Jacobson K, Hou Y, Wojcieszyn J: Exp Cell Res 116:179–189, 1978.
25. Wolf D, Dragsten P: personal communication.
26. Schlessinger J, Axelrod D, Koppel DE, Webb WW, Elson EL: Science 195:307–309, 1977.
27. Schlessinger J, Webb WW, Elson EL, Metzger H: Nature 264:550–552, 1976.
28. Schlessinger J, Barak LS, Hammes GG, Yamada KM, Pastan I, Webb WW, Elson EL: Proc Natl Acad Sci USA 74:2909–2913, 1977.
29. Axelrod D, Ravdin P, Koppel DE, Schlessinger J, Webb WW, Elson EL, Podleski TR: Proc Natl Acad Sci USA 73:4594–4598, 1976.
30. Schlessinger J, Shechter, Y, Cautrecasas P, Willingham MC, Pastan I: Proc Natl Acad Sci USA 75:5353–5357, 1978.
31. Fahey PF, Webb WW: Biochemistry 17:3046–3053, 1978.
32. Poo MM, Cone RA: Nature 247:438–441, 1974.
33. Saffman PG, Delbruck M: Proc Natl Acad Sci USA 72:3111–3113, 1975.
34. Elson EL, Schlessinger J: In Schmitt FO and Worden FG (eds): "The Neurosciences, Fourth Study Program." Cambridge, Massachusetts: MIT Press, 1979, pp 691–701.
35. Koch GLE, Smith MJ: Nature 273:274–278, 1978.
36. Flanagan J, Koch GLE: Nature 273:278–281, 1978.
37. Albertini DF, Berlin RD, Oliver JM: J Cell Sci 26:57–75, 1977.
38. Bretscher A, Weber K: Proc Natl Acad Sci USA 76:2321–2325, 1979.
39. Ash JF, Louvard D, Singer SJ: Proc Natl Acad Sci USA 74:5584–5588, 1977.
40. Geiger B: Cell 18:193–205, 1979.
41. Wu ES, Jacobson K, Szoka F, Portis A: Biochemistry 17:5543–5550, 1978.
42. Darszon A, Vandenberg CA, Ellisman MH, Montal M: J Cell Biol 81:446–452, 1979.
43. Racker E: J Supramol Struct 6:215–228, 1977.
44. Wolf DE, Schlessinger J, Elson EL, Webb WW, Blumenthal R, Henkart P: Biochemistry 16:3476–3483, 1977.
45. Smith LM, Parce JW, Smith BA, McConnel HM: Proc Natl Acad Sci USA 76:4177–4179, 1979.
46. Johnson M, Edidin M: Nature 272:448–450, 1978.
47. deLaat SW, Van der Saag PT, Elson EL, Schlessinger J: Proc Natl Acad Sci USA (in press).
48. Reidler J, Wiegandt H, Schlessinger M, Elson EL: in preparation.
49. Edelman GM: Science 192:218–226, 1976.
50. Condeelis J: J Cell Biol 80:751–758, 1979.
51. Ben-Ze'ev A, Duerr A, Solomon F, Penman S: Cell 17:859–865, 1979.
52. Schlessinger J, Elson EL, Webb WW, Yahara I, Rutishauser U, Edelman GM: Proc Natl Acad Sci USA 74:1110–1114, 1977.