Biochimica et Biophysica Acta 896 (1987) 57–63
Elsevier

BBA 73175

The mobility of concanavalin A receptors and surface immunoglobulins on rat hepatocyte plasma membranes

György Lustyik *, Kenichi Kitani ** and Minoru Ohta

Tokyo Metropolitan Institute of Gerontology, First Laboratory of Clinical Physiology, 35-2 Sakaecho, Itabashi-ku, Tokyo 173 (Japan)

(Received 3 September 1986)

Key words: Cell surface; Concanavalin A receptor; Protein mobility; Photobleaching recovery; FRAP; Aging; (Rat liver)

Lateral mobilities of lectin receptors and surface immunoglobulins were measured in plasma membranes of hepatocytes prepared by smearing small pieces of rat liver tissue and then using the fluorescence recovery after photobleaching (FRAP) technique. Smears were treated with various doses of fluorescein isothiocyanate (FITC) conjugated concanavalin A (ConA), succinylated ConA (SConA), wheat germ agglutinin (WGA), and soybean agglutinin (SBA), as well as with rabbit anti-rat IgG (RARa/IgG) and goat anti-rat IgM(Fc) (GARa/IgM(Fc)) antisera. 10 μg/ml ConA and SConA concentrations and a 55 × dilution of the GARa/IgM(Fc) antiserum were found to be suitable for measuring the lateral mobilities dependent on age. Diffusion constant and mobile fractions of receptor complexes were measured in different age groups of female Fisher rats (from 1 to 26 month-old). The FRAP measurements revealed that at least two major receptor sites can be distinguished in cell membranes of compact tissue (similar to the cultured and isolated cells), forming a mobile and an immobile fraction. The mobile fractions of both the lectin receptors and the surface immunoglobulins tended to decrease with age, while the age differences of the diffusion constants were not statistically significant. The observed alterations could be due to the covalent crosslinking of the mobile receptors to immobile patches and/or to the retardation of free diffusion by the cytoskeleton, dependent on age.

Introduction

Since the redistribution of macromolecules in the intramembrane plane was first demonstrated by fusing together mouse and human cultured cell lines and the spreading of the surface antigens observed on the fused heterocaryons [1], the lateral mobility of many membrane proteins has been clearly established in wide variety of cells. A very effective optical technique, fluorescence recovery after photobleaching (FRAP), is the most widely used method for measuring lateral mobilities in both living cells and model systems [2–8]. Attention has been focused predominantly on three areas: (1) quantitation of the observed mobilities in terms of diffusion constant (D) and fractional recovery (R) [9–14]; (2) revealing the importance of the protein movement in determining the functional properties of the cell membranes [15–19] and (3) gaining insight into factors which control

* Dr. G. Lustyik was the awardee of the visiting scientist scholarship from the Tokyo Metropolitan Institute of Gerontology.

** To whom reprint requests should be addressed.

Correspondence: Dr. G. Lustyik, Fritz Verzar International Laboratory for Experimental Gerontology (VILEG), Hungarian Section, University Medical School, H-4012 Debrecen, Hungary.

the mobility of membrane components [20–26].

Experimental data accumulated for mammalian cells showed several common characteristics of the lateral diffusion of membrane proteins. The two most important observations in plasma membrane have been that: (1) the diffusion constant of diffusible membrane proteins is considerably lower than expected theoretically from the hydrodynamic model of Saffman and Delbruck [27] and also lower than that measured with proteins reconstituted into lipid bilayers; (2) the diffusible fraction (R) of proteins is generally less than 100%, and certain proteins are virtually nondiffusible (an exception being rhodopsin in the amphibian rod outer segment disk [28]).

Most of our knowledge of the mobility characteristics of membrane proteins is derived from observations on cultured or isolated cells. However, it is important to assess the extent to which these results can be extrapolated to parenchymal cells of compact tissues, like liver, muscle, nervous system, etc. One of the problems that needs to be solved in this context is that diffusion measurements on living cells of compact tissue require special preparation methods. By virtue of the findings reported in the papers of Zs.-Nagy et al. [29,30] we tried to circumvent the preparation problem in rat hepatocytes by using exogenous markers in smeared liver specimens.

The present study is concerned with the age dependence of the translational movement of membrane proteins of rat liver cells. Hepatocytes were labeled with concanavalin A (ConA) and succinylated concanavalin A (SConA) lectins and goat anti-rat IgM(Fc) antiserum conjugated with fluorescein isothiocyanate (FITC). We chose lectin receptors and surface immunoglobulins for our first investigation on smeared samples with exogenous ligands because their properties are well characterized in isolated and cultured cells. It was expected that membrane damage, predominantly the accumulation of covalent crosslinks between membrane components during aging, would retard the diffusion in the cell membrane. The results demonstrate that under certain conditions the smearing technique can be useful for studies of protein mobilities in plasma membrane of cells of solid tissues, like the liver.

Materials and Methods

General. Female Fisher 344 rats (Japan, Charles River Astugi) were maintained in the institute's SPF aging farm on a standard diet (CRF, Oriental, Tokyo) with free access to acidified water (pH 2.5–3.0, residual chlorine 10 ppm). Their survival and other conditions are described elsewhere [31]. The animals were killed by decapitation and hepatocytes were prepared from the freshly removed livers (usually within one minute subsequent to the removal) by smearing small tissue pieces between two slides. In order to minimize the cell damage during smearing and to standardize the method we used a preparation procedure similar to that described in detail recently [29,30], with the exceptions that non-frosted slides were used in order to improve the cell recognition, and 50 μm thick plastic tape was used on the smearing slide only, to avoid any influence of the tape components on the smears. The smears were then incubated with the FITC-labeled lectins and immunoconjugates as detailed below.

Two series of experiments were performed. First, four labeled lectins, concanavalin A (ConA-FITC), succinyl concanavalin A (SConA-FITC), wheat germ agglutinin (WGA-FITC) and soybean agglutinin (SBA-FITC) as well two immunoconjugates, FITC-labeled rabbit anti-rat IgG (RARa/IgG-FITC) and goat anti-rat IgM(Fc) (GARa/IgM(Fc)-FITC) antisera, were applied at various concentrations to the incubating solution. The purpose of these 'preliminary' experiments was to establish the optimal label concentrations for the measurement of protein mobility dependent on age.

In the second series of experiments different age groups of female rats (from one to 26 month-old) were used as test animals. Lateral mobility of membrane proteins of liver cells was measured after labeling the cells with ConA-FITC, SConA-FITC and GARa/IgM(Fc)-FITC (see below).

Materials. ConA-FITC and SConA-FITC were obtained from Sigma (U.S.A.). WGA-FITC, SBA-FITC and RARa/IgG-FITC was from Miles Laboratories (Israel). GARa/IgM(Fc)-FITC was purchased from Nordic (The Netherlands).

Incubation and slide preparation. The incubating solutions were prepared with Krebs-Ringer bi-

carbonate buffer (pH 7.4) [32]. The prepared smears were covered immediately with a 200 μl solution containing the FITC conjugated label and incubated for 10 min at 37°C. They were then washed intensively with the buffer saline, and wet mounted and sealed with paraffin in order to avoid drying during the fluorescence recovery experiments.

In preliminary experiments the following concentrations were tested:
ConA-FITC: 5, 10, 20 and 50 μg/ml.
WGA-FITC: 1, 2, 5 and 10 μg/ml.
SBA-FITC: 1, 5, 10, 50, 100 and 200 μg/ml.
RARa/IgG-FITC: 11x, 22x, 55x and 110x dilution of the antiserum.
GARa/IgM(Fc)-FITC: 11x, 22x, 55x and 110x dilution of the antiserum.

In the experiments in which the lateral mobility of membrane proteins were measured in relation to rat age, 10 μg/ml ConA-FITC and SConA-FITC and 55x dilution of the GARa/IgM(Fc)-FITC were used, these concentrations having been found to be optimal for labeling liver cells.

Fluorescence recovery after photobleaching. Diffusion coefficients and recoveries (immobile fractions) were determined by the FRAP technique using the instrument described in detail by Zs.-Nagy et al. [29]. Attenuated laser beam ($\lambda = 476.5$ nm) from a NEC GLG 3200 argon ion laser was focused on the cell membrane through the objective (FLPL 40, NA = 0.75) of a phase contrast/epifluorescence microscope. The approximately gaussian beam was focused to 1.5 μm of its $1/e^2$ radius. A beam splitting system consisting of two 10 mm thick optical flats (Melles Griot 02 FQD 007 type) which produced an approximately 10^4-fold attenuation of the full laser beam (100–150 mW). The unattenuated beam could be turned on and off by means of a shutter which allowed bleaching pulses greater than 10 ms to be used.

Approximately 50–60% of the FITC in the illuminated area was irreversibly bleached by a short (100–200 ms) pulse of the unattenuated beam created by opening the shutter. The fluorescence of the bleached spot, as well as the prebleaching fluorescence were followed with a Hamamatsu R649 type photomultiplier operated in the photon counting mode at −20°C. Typical

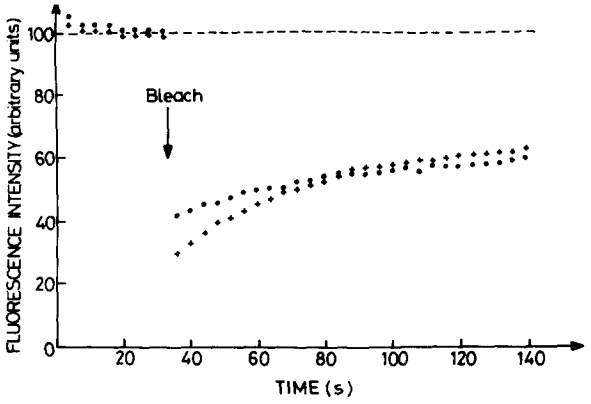

Fig. 1. Two typical photobleaching recovery curves: ConA-FITC bound to hepatocyte membrane of an old rat (●) and GARa/IgM(Fc)-FITC bound to the liver cell membrane of a young animal (+). The diffusion constants were $1.3 \cdot 10^{-10}$ cm^2/s and $1.5 \cdot 10^{-10}$ cm^2/s, respectively. Mobile fractions were 43% and 69%.

recovery curves are shown in Fig. 1. Fluorescence was measured in a duty circle of 1 s every 4 s. Intensity signals were applied to a Hamamatsu C 1230 type photon counter providing digital data of the intensities for the on line NEC PC-9801E computer. The data were converted into diffusion coefficients and percent recoveries by using the calculation procedure described by Yguerabide et al. [33].

Prepared specimens were examined using the microscope's phase contrast mode. The overall fluorescence of the cell membranes could be observed under epi-illumination using the completely defocused laser beam. Monolayered cells showing intact morphology and homogeneous fluorescent labeling were chosen for measurements. Only a single bleach was applied to each of the measured cells. The FRAP measurements started immediately after the specimen preparation and continued for approximately 90–110 min. All the measurements were performed at 25–26°C.

Results

Concentration dependence of the labeling

The phase contrast microscopic picture of the liver smear showed that mononuclear hepatocytes were the predominant form among the morphologically intact cells. The cells displayed a rela-

tively weak labeling with ConA-FITC and SConA-FITC. Sufficiently high FITC fluorescence intensity for FRAP measurements could be obtained only with concentrations higher than 5 μg/ml of the incubating medium. The ConA receptors were generally uniformly distributed without any cap-like asymmetrical arrangement.

Both the diffusion constant and the mobile fraction varied depending on the concentration of the probe (Fig. 2). The recovery decreased to about 20% with ConA concentrations above 20 μg/ml. Since at concentrations of 5 μg/ml or below the intensity was too low to allow an appropriate measurement, 10 μg/ml ConA-FITC and SConA-FITC concentrations of the incubating media were chosen for the measurement of the lateral mobility of ConA receptor complexes dependent on age.

A homogenous, sufficiently intensive labeling was obtained with WGA-FITC lectin. Unlike smear preparations labeled with the other two

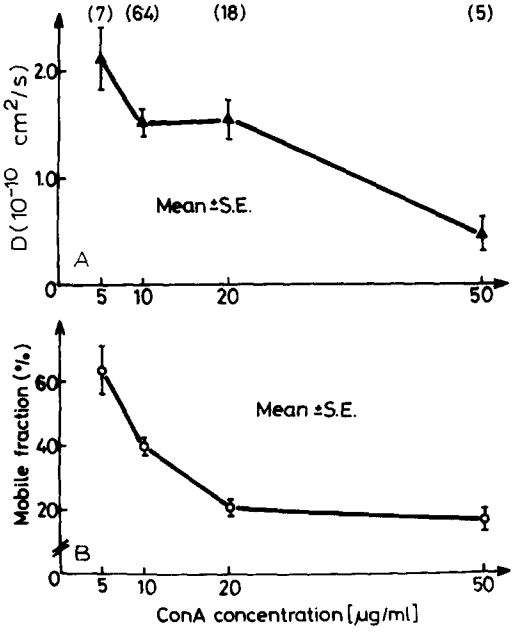

Fig. 2. Diffusion coefficients (▲) of ConA receptors and mobile fractions (O) as measured on rat liver cells incubated with various doses of ConA-FITC in smear. Concentrations below 5 μg/ml did not provide appropriate fluorescent labeling. The data were obtained from measurements on the number of cells indicated in parentheses.

TABLE I

LATERAL MOBILITY OF ConA RECEPTORS IN THE PLASMA MEMBRANE OF HEPATOCYTES

Labeling with 10 μg/ml ConA-FITC. D and R values are presented as means ± S.E. Abbreviations: ANOVA, one-way analysis of variance; M.Sqr., mean squares; df, degree of freedom (numerator, denominator); n.s., not significant.

Age of rats [a]	Diffusion constant (D) (10^{-10} cm^2/s)	Mobile fraction (R) (%)
1 month (40)	1.95 ± 0.13	50.5 ± 1.8
2.5 months (39)	1.74 ± 0.10	49.8 ± 2.0
6 months (61)	1.97 ± 0.12	50.4 ± 1.7
12 months (48)	1.86 ± 0.12	50.3 ± 1.7
17.5 months (52)	1.68 ± 0.14	45.3 ± 1.6
23.5 months (65)	1.66 ± 0.07	43.7 ± 1.4

ANOVA:		
M. Sqr. between groups:	102.46	440.15
within groups:	62.79	125.48
F:	1.632	3.508
df (num, denom):	5, 299	5, 299
Significance:	n.s. ($P > 0.1$)	$P < 0.005$

[a] In parentheses: number of measured cells.

TABLE II

LATERAL MOBILITY OF SUCCINYL-CONCANAVALIN A BOUND TO HEPATOCYTES IN LIVER SMEAR

Labeling with 10 μg/ml SConA-FITC. D and R values are presented as means ± S.E. Abbreviations: ANOVA, one-way analysis of variance; M. Sqr., mean squares; df, degree of freedom (numerator, denominator).

Age of rats [a]	Diffusion constant (D) (10^{-10} cm^2/s)	Mobile fraction (R) (%)
1 month (40)	2.43 ± 0.17	60.4 ± 2.1
2.5 months (49)	2.82 ± 0.14	61.8 ± 2.0
4.5 months (18)	2.03 ± 0.21	58.7 ± 3.8
6 months (45)	1.91 ± 0.11	49.4 ± 2.0
7.5 months (51)	2.04 ± 0.13	56.4 ± 2.2
12 months (51)	1.85 ± 0.14	59.2 ± 2.2
17.5 months (48)	1.80 ± 0.10	51.1 ± 1.7
23.5 months (29)	2.09 ± 0.13	46.0 ± 2.5

ANOVA:		
M. Sqr. between groups:	561.13	1304.83
within groups:	78.09	202.18
F:	7.185	6.545
df (num, denom):	7, 323	7, 323
Significance:	$P < 0.005$	$P < 0.005$

[a] In parentheses: number of measured cells.

TABLE III

LATERAL MOBILITY OF GARa/IgM(Fc)-FITC IMMUNOGLOBULINS BOUND TO HEPATOCYTES IN LIVER SMEAR

D and *R* values are presented as means ± S.E. Abbreviations: ANOVA, one-way analysis of variance; M.Sqr., mean squares; df, degree of freedom (numerator, denominator); n.s., not significant.

Age of rats [a]	Diffusion constant (D) (10^{-10} cm^2/s)	Mobile fraction (R) (%)
1 month (20)	1.69 ± 0.20	67.2 ± 2.5
2.5 months (15)	1.79 ± 0.23	62.8 ± 2.3
17 months (14)	1.91 ± 0.20	54.8 ± 3.0
26 months (23)	1.44 ± 0.16	59.0 ± 3.6
ANOVA:		
M.Sqr. between groups:	107.01	480.33
within groups:	67.18	173.32
F:	1.593	2.771
df (num, denom):	3, 68	3, 68
Significance:	n.s. ($P > 0.1$)	$P < 0.025$

[a] In parentheses: number of measured cells.

lectins both the plasma membrane and the cell nuclei in 30–40% of the liver cells showed fluorescence after incubation. The percentage of cells showing nucleus fluorescence increased with incubation time. The fluorescence intensity of nuclei exceeded the intensity of plasma membrane. The diffusion coefficients and mobile fractions measured in the nucleus membrane of these cells were found to be significantly lower than those recently reported for isolated nuclei by Schindler et al. [34].

Since labeling of nuclei membrane can be the result of a certain type of plasma membrane damage resulting in an increased permeability for macromolecules, we did not extensively study WGA-FITC. The lateral mobility of WGA receptors of cell membrane, however, showed a concentration dependence similar to the ConA receptors.

When SBA-FITC was used as a cell label no measurable fluorescence was obtained even at higher incubating concentrations (up to 250 μg/ml) and longer incubation times (2 h). The RARa/IgG-FITC produced a mottled fluorescence pattern on hepatocytes membranes at all concentrations (dilutions) of the antiserum which were used. No FRAP measurement was performed with either the SBA-FITC or RARa/IgG-FITC probes.

The incubation of liver smears with GARa/IgM(Fc)-FITC resulted in a homogeneous labeling of the cell surface. The lateral mobility of the fluorescent complexes as well as the mobile fraction showed a clear concentration dependence.

Lateral mobility dependent on age

The diffusion constant as well as the percent recovery of ConA receptor complexes labeled with ConA-FITC and SConA-FITC and surface immunoglobulins are shown in Table I, II and III, respectively. The results indicate, that the diffusion constants of the ConA and the GARa/IgM(Fc) did not change significantly with age, although the D of the ConA receptors tended to decrease over time. The diffusion constant of the SConA was significantly higher in the young (1 and 2.5 month-old) animals than in the older. A greater difference can be seen among the age-groups in the mobile fraction of the receptors. The mobile fractions of both lectins and GARa/IgM(Fc) were considerably lower in the 23.5–26 month-old rats than in the young ones. The diffusion constants and the mobile fractions of the individual cells were scattered over a wide range.

Discussion

Since increasing ConA doses and longer incubation times were reported to reduce the diffusion constant and the mobile fraction [35], we used the lowest possible lectin concentrations for cell labeling. The lectins showed a relatively weak binding to the surface receptors of rat liver cells in smears. In our experiments the 10 μg/ml ConA (and SConA) concentration was found to be the lowest concentration at which a relatively stable and measurable green FITC fluorescence could be observed. Others have stained hepatocytes with both fluorescein and peroxydase labeled lectins using lectin concentrations similar to or higher than that applied in the present experiments [36–38].

Photobleaching measurements reported in the present study demonstrated that in young (1 and

2.5 month-old) animals both the diffusion constant and the mobile fraction of the lectin receptors were lower in the plasma membrane of liver cells treated with ConA than in the SConA-treated cells. This agrees with previous reports demonstrating a greater decrease for the tetravalent ConA than for its succinylated derivative [35,39].

The $(1.5–2.8) \cdot 10^{-10}$ cm^2/s values of the diffusion coefficients of lectin receptors on liver cells found in our study are larger than generally measured on other cell membranes [2,21,35,40]. On the other hand, the present results are comparable with the receptor mobilities in normal and virus transformed mouse fibroblasts [41] and even higher diffusion coefficients were calculated from electrophoretic measurements on embryonic muscle cells [42]. Diffusion constants of receptors labeled with SConA fell into the same range as that of the unfertilized and fertilized mouse eggs [15]. It is noteworthy that we obtained relatively low fractional recoveries: in ConA treated hepatocytes 40–50%, and in SConA-treated ones 45–60% of the receptors was mobile. This may be due to: (1) the receptors exist in at least two states on hepatocytes, forming a mobile and an immobile fraction or (2) lectin labeling itself caused two apparently different receptor states.

Basically, the same conclusion can be drawn for the lateral mobility of surface antigens. The diffusion coefficient and the mobile fraction of both the lectin receptors and surface immunoglobulins strongly depended upon the dose of the probe, most probably due to the aggregation of the receptor complexes. Since ConA is rather tetravalent in the circumstances of our labeling procedure [43] crosslinking can produce impermeable (but fluorescently invisible) patches forming an 'archipelago' on the cell membrane, and consequently reduce the diffusion coefficient [44]. On the other hand, links between originally mobile and immobile complexes may reduce the mobile fraction. Thus, our results suggest that none of ligands used in the present study is ideal for the purpose of FRAP measurement, although we could obtain some information on the hepatocyte plasma membrane that was not accessible up to now.

In our previous work using autofluorescence [30] we observed a significantly linear decrease of the diffusion constant of membrane proteins in relation to age, and much higher recoveries (> 80% even in old rats). The results obtained from the present measurement tends to be similar to our previous work but the age differences are less clear. Discovery of the real causes for the differences between the two studies using exogenous and endogenous fluorescent markers awaits future work.

Although the results presented in this paper do not offer a final answer to the behavior of the surface receptors of liver cells, the acceptable agreement with previous experimental results suggests that the most important properties of the receptors are preserved during the smearing and the preparation. Moreover, the results indicate the utility of the FRAP technique for research on rat liver cells.

Acknowledgements

This study was supported in part by Grants in Aid from the Ministry of Science and Technology of Japan and from the Tokyo Metropolitan Institute of Gerontology 'The pharmacodynamics in the elderly' and from the Life Science Foundation. G. Lustyik was the awardee of a visiting scientist scholarship from the Tokyo Metropolitan Institute of Gerontology.

References

1 Frye, L.D. and Edidin, M. (1970) J. Cell. Sci. 7, 319–336
2 Cherry, R.J. (1979) Biochim. Biophys. Acta 559, 289–327
3 Axelrod, D. (1983) J. Membrane Biol. 75, 1–10
4 Lauffenburger, D. and DeLisi, C. (1983) Int. Rev. Cytol. 84, 269–302
5 Shinitzky, M. and Henkart, P. (1969) Int. Rev. Cytol. 60, 121–147
6 Nicolson, G.L., Poste, G. and Ji, T.H. (1977) in Dynamic Aspects of Cell Surface Organization pp. 1–73, North-Holland, Amsterdam
7 Vaz, W.L.C., Criado, M., Madeira, V.M.C., Schoellmann, G. and Jovin, T.M. (1982) Biochemistry 21, 5608–5612
8 Criado, M., Vaz, W.L.C., Barrantes, F.J. and Jovin, T.M. (1982) Biochemistry 21, 5750–5755
9 Axelrod, D., Koppel, D.E., Schlessinger, J., Elson, E.L. and Webb, W.W. (1976) Biophys. J. 16, 1055–1069
10 Koppel, D.E., Axelrod, D., Schlessinger, J., Elson, E.L. and Webb, W.W. (1976) Biophys. J. 16, 1315–1329
11 Koppel, D.E. (1979) Biophys. J. 28, 281–292
12 Koppel, D.E. and Sheetz, P.M. (1983) Biophys. J. 43, 175–181

13 Jacobson, K., Derzko, Z., Wu, E.-S., Hou, Y. and Poste, G. (1976) J. Supramol. Struct. 5, 565–576
14 Davoust, J., Devaux, P.F. and Leger, L. (1982) Eur. Mol. Biol. Organ. J. 1, 1233–1238
15 Wolf, D.E. and Ziomek, C.A. (1983) J. Cell Biol. 96, 1786–1790
16 Aizenbud, B.M. and Gershon, N.D. (1982) Biophys. J. 38, 287–293
17 Peters, R. (1981) Cell. Biol. Int. Rep. 5, 733–760
18 Golan, D.E. Alecid, M.R., Veatch, W.R. and Rando, R.R. (1984) Biochemistry 23, 332–339
19 Damjanovich, S., Trón, L., Szöllősi, J., Zidovetzki, R., Vaz, W.L.C., Regateiro, F., Arndt-Jovin, D. and Jovin, T.M. (1983) Proc. Natl. Acad. Sci. USA 80, 5985–5989
20 Wu, E.-S., Tank, D.W. and Webb, W.W. (1982) Proc. Natl. Acad. Sci. USA 79, 4962–4966
21 Schlessinger, J., Elson, E.L., Webb, W.W., Yhara, I., Rutishauser, U. and Edelman, G.M. (1977) Proc. Natl. Acad. Sci. USA 74, 1110–1114
22 Henis, Y.I. and Elson, E.L. (1981) Proc. Natl. Acad. Sci. USA 78, 1072–1076
23 Tank, D.W., Wu, E.-S. and Webb, W.W. (1982) J. Cell Biol. 92, 207–212
24 McCloskey, M. and Poo, M.-M. (1984) Int. Rev. Cytol. 87, 19–81
25 Jacobson, B.S. (1983) Tissue Cell 15, 829–852
26 Pink, D.A. (1985) Biochim. Biophys. Acta 818, 200–204
27 Saffman, P.G. and Delbruck, M. (1975) Proc. Natl. Acad. Sci. USA 72, 3111–3113
28 Poo, M.-M. and Cone, R.A. (1974) Nature (Lond.) 247, 438–441
29 Zs.-Nagy, I., Ohta, M., Kitani, K. and Imahori, K. (1984) Mikroskopie (Wien) 41, 12–25
30 Zs.-Nagy, I., Kitani, K., Ohta, M., Zs.-Nagy, V., and Imahori, K. (1986) Arch. Gerontol. Geriatr. 5, 131–146
31 Nokubo, M. (1985) J. Gerontol. 40, 409–414
32 Umbreit, W.W., Burris, R.H. and Stauffer, J.F. (eds.) (1964) Manometric Techniques, pp. 1932, Burges, Minneapolis
33 Yguerabide, J., Schmidt, J.A. and Yguerabide, E.E. (1982) Biophys. J. 40, 69–75
34 Schindler, M., Holland, J.F. and Hogan, M. (1985) J. Cell Biol. 100, 1408–1414
35 Schlessinger, J., Koppel, D.E., Axelrod, D., Jacobson, K., Webb, W.W. and Elson, E.L. (1976) Proc. Natl. Acad. Sci. USA 73, 2409–2413
36 Roth, J., Neupert, G. and Block, F. (1975) Exp. Pathol. 10, 143–155
37 Roth, J. and Thoss, K. (1975) Exp. Pathol. 10, 258–267
38 Roth, J., Neupert, G. and Thoss, K. (1975) Exp. Pathol. 10, 309–317
39 Edelman, G.M. (1976) Science 192, 218–226
40 Metcalf, T.N., Wang, J.L., Schubert, K.R. and Schindler, M. (1983) Biochemistry 22, 3969–3975
41 Eldridge, C.A., Elson, E.L. and Webb, W.W. (1980) Biochemistry 19, 2075–2079
42 Poo, M.-M., Poo, W.-J.H. and Lam, J.W. (1978) J. Cell Biol. 76, 483–501
43 Senear, D.F. and Teller, D.C. (1981) Biochemistry 20, 3076–3083
44 Saxton, M.J. (1982) Biophys. J. 39, 165–173