LOCALIZATION OF CELL MEMBRANE COMPONENTS BY SURFACE DIFFUSION INTO A "TRAP"

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ABSTRACT Randomly distributed cell membrane components may become localized toward a specific region of the surface as a result of cell-cell contact or the cell's exposure to extracellular ligands. The mechanism for this localization process is unknown. In the present study, we investigated the plausibility of a passive mechanism, namely that a local region of the cell surface serves as a "trap" for diffusing membrane proteins. Based on a model of spherical cell with a single circular trap on the surface, we derived the equations describing the surface density distribution and the average lifetime of the trappable molecules in the trap-free region of the membrane. This surface-trap theory was then used to analyze our experimental finding on the rapid localization of muscle surface soybean agglutinin receptors induced by cell-cell contact in culture. The result indicates that the rate of localization of these receptors toward the cell-cell contact site can be accounted for by assuming that the receptors possess a diffusion coefficient of about 2.5×10^{-9} cm^2/s (range: 1.2–9.3 $\times 10^{-9}$ cm^2/s) before they are trapped at the contact site. Independent measurement of the rate of lateral diffusion of these receptors yielded a lateral diffusion coefficient of about 1.9×10^{-9} cm^2/s (range: 1.2–2.7 $\times 10^{-9}$ cm^2/s), a value within the range of that predicted by the rate of localization. We thus conclude that lateral diffusion of mobile membrane components toward a local trap is a plausible mechanism for their localization induced by local surface modulation.

I. INTRODUCTION

One of the current problems in membrane biology is to understand the mechanism(s) by which the topography of membrane components can be modulated or stabilized within the fluid lipid-protein matrix of the cell membrane. A characteristic example of this problem is the localization of certain membrane components toward a specific region of the cell surface. The localization may result from either cell-cell contact (1–3) or the cell's exposure to extracellular ligands (4–7). There are two aspects of the problem of localization in cell membrane: first, the induction of localizing movement, and second, the stabilization of the localized components. The present study focuses on the first aspect, namely, a mechanism by which pre-existing, uniformly distributed membrane components could become localized in a specific region of the cell membrane.

Several hypotheses have been proposed to account for the induction of localizing movements (8–13). They can be divided into two broad categories: (*a*) active translocation of membrane components through cytoplasmic, energy-requiring processes (8–11); and (*b*) passive spontaneous redistribution of the membrane components as the result of the surface modulation of the components (12,13). In the present paper, we will demonstrate that if surface modulation results in the formation of a local surface "trap" for certain membrane

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components, passive lateral diffusion of the particular component into the trap could lead to an efficient localization of the component. In the following sections, we first present theoretical calculations of the surface density distribution of mobile "trappable" membrane proteins in the trap-free region of a spherical cell after the onset of the trapping process and the average lifetime of the proteins in the trap-free region of the membrane. We then describe our experimental findings on the localization of muscle surface glycoproteins induced by cell-cell contact and the rate of lateral diffusion of these proteins. Finally, the surface trap theory presented earlier will be used to analyze these experimental findings. A similar trap model has been proposed previously by Edwards and Frisch (14) for the localization of acetylcholine receptors at the muscle endplate.

II. THEORY

A Surface Trap Model Analysis

The present analysis is based on a simple model depicted in Fig. 1. The cell membrane is assumed to be a perfect spherical membrane of radius r_0. The trappable membrane proteins are represented as homogeneous particles embedded in the membrane, free to undergo lateral diffusion in the plane of the membrane with a diffusion coefficient D. At time equal to zero, the surface area within convergence angle $2\theta_0$ becomes a perfect trap for the particles, i.e., no diffusion out of the boundary of the trap is allowed. The following analysis aims to determine the surface density (C) of the particle, as a function of angle θ and time t. For mathematical simplicity, both the trap and the diffusion process are assumed to possess azimuthal symmetry.

The equation of diffusion describing the surface distribution of the particle in polar coordinates is given by

$$\frac{\partial C}{\partial t} = \frac{D}{r_0}\frac{\partial}{\partial x}\left[(1 - x^2)\frac{\partial C}{\partial x}\right] \tag{1}$$

where $x = \cos\theta$, and $C = C(\theta,t)$. Eq. 1 satisfies the initial condition of uniform surface density, i.e., $C(\theta,0) = C(x,0) = C_0$, where $x_0 < x \leq 1$ and C_0 is the constant surface density. The boundary condition that reflects the perfect trap model can be written as

$$C(x_0, t) = 0$$

where $x_0 = \cos(\pi - \theta_0) = -\cos\theta_0$. This boundary condition is mathematically identical to the situation of a perfect "sink," where all particles that diffuse into the boundary of the cone defined by θ_0 will

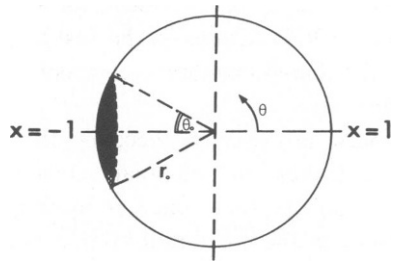


FIGURE 1 Model of a surface trap on a spherical membrane. A surface trap enclosed by a convergence angle of 2 θ_0 is represented by a shaded circular cap. The trappable molecules are free to undergo lateral diffusion in the plane of the membrane except within the trap, $x = \cos\theta$, and r_0 is the radius of the spherical membrane.

disappear from the pool of diffusible particles. In our trap model, these trapped particles are considered to be a separate, immobile fraction of the total population of particles. The trap can thus be considered as possessing zero surface density of mobile particles.

It is well known that the solution of the diffusion equation in spherical polar coordinates involves the use of Legendre functions. Similar to the treatments of electric potentials with axial symmetry and conical boundary conditions (15,16), the use of real and nonintegral Legendre functions are required in the present problem to express the general solution of Eq. 1 in the following form:

$$C(x, t) = \sum_{\nu} A_\nu P_\nu(x) \exp\left[-\frac{D}{r_0^2}\nu(\nu + 1)t\right] \tag{2}$$

where ν is real but not an integer. $P_\nu(x)$ can be denoted by the Gaussian hypergeometric functions (16,17):

$$P_\nu(x) = {}_2F_1\left(-\nu, \nu + 1; 1; \frac{1-x}{2}\right) \tag{3}$$

and

$${}_2F_1(a, b; c; z) = 1 + \frac{ab}{c}\frac{z}{1!} + \frac{a(a + 1)b(b + 1)}{c(c + 1)}\frac{z^2}{2!} + \cdots\cdots$$

The boundary condition $C(x_0,t) = 0$ can be rewritten as:

$$\mathrm{P}_{\nu_s}(x_0) = 0 \tag{4}$$

where ν_s ($s = 0, 1, 2, \ldots$) are successive real numbers for which Legendre function $P_{\nu_s}(x_0)$ vanishes.

The orthogonality and completeness of the functions $P_{\nu_s}(x)$ in the interval $x_0 \le x \le 1$ can be shown in the same fashion as for integral Legendre functions $P_\ell(x)$, where $\ell = 0, 1, 2 \ldots$ (16). Following Hall (15), we define:

$$\int_{x_0}^{1} P_{\nu_s}(x)P_{\nu_t}(x)\,dx = H_s(x_0)\delta_{st} \tag{5}$$

and

$$\int_{x_0}^{1} P_{\nu_s}(x)\,dx = I_s(x_0). \tag{6}$$

The initial condition $C(x,0) = C_0$, where $x_0 < x \le 1$, can thus be described as

$$\sum_{s=0}^{\infty} \mathrm{A}_s\mathrm{P}_{\nu_s}(x) = C_0$$

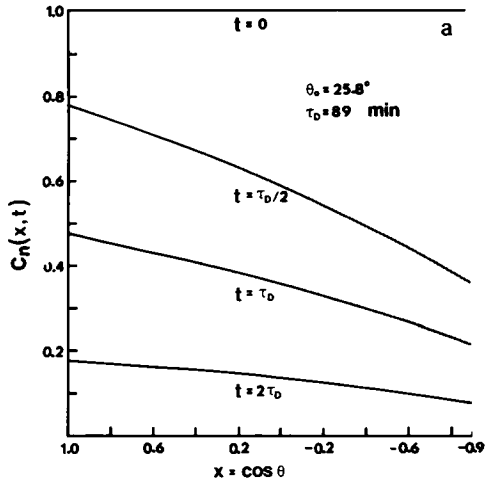
and from Eqs. 5 and 6, we obtain

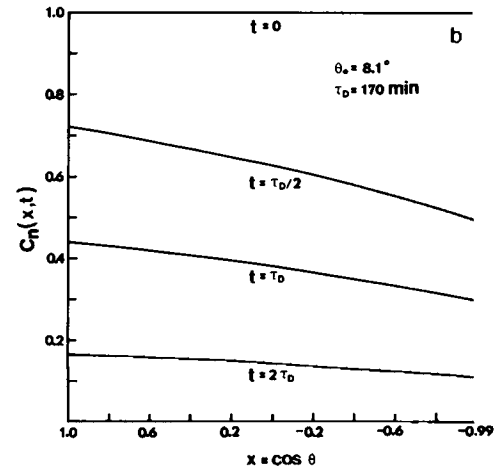
$$A_s = \frac{I_s(x_0)}{H_s(x_0)}C_0.$$

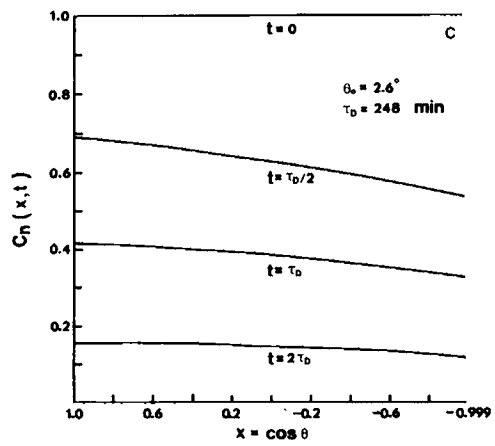
The solution for the surface density of the particles is thus given by

$$C(x,t) = C_0 \sum_{s=0}^{\infty} \frac{I_s(x_0)}{H_s(x_0)} P_{\nu_s}(x) \exp\left[-\frac{D}{r_0^2}\nu_s(\nu_s + 1)t\right] \tag{7}$$

where $P_{\nu_s}(x_0) = 0$, and the values of ν_s, $H_s(x_0)$, as well as $I_s(x_0)/H_s(x_0)$ can be obtained from the graphs


t = 0
a
θ₀ = 25.8°
τD = 89 min
t = τD/2
t = τD
t = 2τD
Cn(x,t)
1.0
0.8
0.6
0.4
0.2
1.0 0.6 0.2 -0.2 -0.6 -0.9
X = COS θ


t = 0
b
θ₀ = 8.1°
τD = 170 min
t = τD/2
t = τD
t = 2τD
Cn(x,t)
1.0
0.8
0.6
0.4
0.2
1.0 0.6 0.2 -0.2 -0.6 -0.99
X = COS θ


t = 0
c
θ₀ = 2.6°
τD = 248 min
t = τD/2
t = τD
t = 2τD
Cn (x,t)
1.0
0.8
0.6
0.4
0.2
1.0 0.6 0.2 -0.2 -0.6 -0.999
X = COS θ

and tables in Hall's report (15). In Fig. 2, we plot $C(x,t)$ in normalized form: $C_n(x,t) = C(x,t)/C_0$, for three different sizes of surface trap with $\theta_0 = 25.8°$, 8.1°, and 2.6° (i.e., $1 + x_0 = 0.1$, 0.01, and 0.001). Three characteristic times of the trapping process: $t = \tau_D/2$, τ_D, and $2\tau_D$ were chosen for the plotting, where $\tau_D = r_0^2/D\nu_0(\nu_0 + 1)$ is the characteristic $1/e$ time for the diffusion process.

From the surface distribution of the diffusing particles, we evaluate the average lifetime of a particle in the diffusion surface ($x_0 < x \leq 1$), by a procedure similar to that developed by Adam and Delbrück (18). The number of particles $P(t)$ remaining in the diffusion surface at time t is given by:

$$P(t) = 2\pi r_0^2 \int_{x_0}^{1} C(x, t)\, dx$$

$$= 2\pi r_0^2 C_0 \sum_{s=0}^{\infty} \frac{[I_s(x_0)]^2}{H_s(x_0)} \exp\left[-\frac{D}{r_0^2}\nu_s(\nu_s + 1)t\right]$$

since the initial condition requires

$$P(0) = 2\pi r_0^2 C_0 \sum_{s=0}^{\infty} \frac{[I_s(x_0)]^2}{H_s(x_0)} = C_0 S_0$$

where

$$S_0 = 2\pi r_0^2 (1 - x_0)$$

is the area of diffusion surface, it follows that:

$$P(t) = P(0) \sum_{s=0}^{\infty} B_s \exp[-t/\tau_s] \tag{8}$$

where

$$B_s = \frac{[I_s(x_0)]^2}{H_s(x_0)(1 - x_0)} \quad \text{and} \quad \tau_s = \frac{r_0^2}{D\nu_s(\nu_s + 1)}.$$

The average lifetime of a particle in the diffusion space, or the average time required for a particle to reach the trap is defined as (18)

$$\bar{\tau} = \int_0^{\infty} dt \cdot t \cdot \frac{d}{dt}\left[1 - \frac{P(t)}{P(0)}\right].$$

Substituting $P(t)$ by Eq. 8, we obtain

$$\bar{\tau} = \sum_{s=0}^{\infty} B_s \tau_s.$$

If we use only the first term of the series, then within a relative error of <1%, the average lifetime of a

FIGURE 2 Normalized surface density distribution $C_n(x, t)$ for a circular surface trap enclosed by a convergence angle of $2\theta_0 (x = \cos\theta$, see Fig. 1). $C_n(x, t) = C(x, t)/C_0$, where C_0 is the initial uniform density of the trappable molecules, τ_D is the characteristic diffusion time of the molecule to reach the trap, as given by $\tau_D = r_0^2/D\nu_0(\nu_0 + 1)$, where r_0 is the radius of the cell, ν_0 is the first real number for which Legendre function $P_{\nu_0}(x_0)$ vanishes (see Eq. 7), and D is the diffusion coefficient of the molecule in the trap-free region of the membrane. (*a*) A trap of angle $\theta_0 = 25.8°$, (*b*) a trap of angle $\theta_0 = 8.1°$, and (*c*) a trap of angle $\theta_0 = 2.6°$. D and r_0 were chosen to be 1.0×10^{-9} cm^2/s and 15 μm, respectively. Note the characteristic diffusion time τ_D increases from 89 to 253 min (a threefold increase) from (*a*) to (*c*), while the surface area of the trap reduces from $0.2\,\pi r_0^2$ to $0.002\,\pi r_0^2$ (a 100-fold reduction, see also Table I).

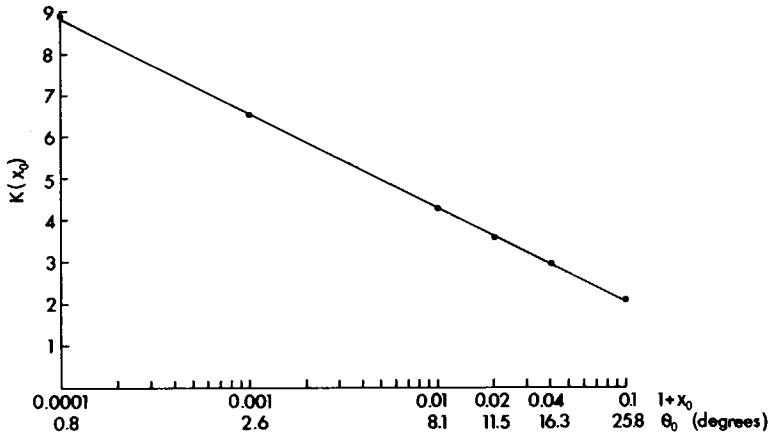


FIGURE 3 Trap geometry function $K(x_0)$, as defined by Eq. 9, is plotted against the trap size (θ_0 = half of the covergence angle enclosing the trap, and $x_0 = -\cos\theta_0$). Data points were calculated according to Eq. 9, using ν_0, $I_0(x_0)$, and $H_0(x_0)$ given by the tables and graphs in the Hall's report (15). The solid line represents a least-square fit to the data points in the range of $2\theta_0$ = 1.6°–51.6°.

particle in the trap-free region of the membrane is given by

$$\bar{\tau} = B_0\tau_0 = \frac{[I_0(x_0)]^2}{H_0(x_0)(1 - x_0)} \frac{r_0^2}{D\nu_0(\nu_0 + 1)}$$

or

$$\bar{\tau} = K(x_0)\frac{r_0^2}{D} \tag{9}$$

where $K(x_0)$ is a trap geometry function determined by the convergence angle ($2\theta_0$) that encloses the trap, $x_0 = -\cos\theta_0$, r_0 is the radius of the cell, and D is the diffusion coefficient of the trappable particles. A plot of $K(x_0)$ vs. $1 + x_0$ is shown in Fig. 3. The points shown were calculated from the values of ν_0, $I_0(x_0)$, and $H_0(x_0)$ given by the tables and graphs of Hall (14). The solid line represents a least-square fit to the data points in the form of

$$K(x_0) = \log_{10}\left[(1 + x_0)^{-2.26}/1.55\right].$$

This simple function of $K(x_0)$ fits satisfactorily the data points for trap size of convergence angle examined (range $2\theta_0$ = 1.6°–51.6°). As an example, we take the radius of a typical cell to be 15 μm, and the diffusion coefficient of a membrane protein to be 1.0×10^{-9} cm²/s, the average lifetimes $\bar{\tau}$ of the protein in the "trap-free" region can be shown to be 76, 1.6×10^2, and 2.5×10^2 min for $1 + x_0 = 0.1$, 0.01, and 0.001, i.e. traps with θ_0 = 25.8°, 8.1°, and 2.6°, respectively (see Table I).

In summary, the theoretical analysis of the surface trap model yielded two results: the surface distribution of the trappable molecules at various times following the onset of trapping process (Eq. 7), and the average lifetime of the molecule in the trap-free region (Eq. 9). Hall's numerical solution of Legendre functions for conical boundary conditions (15) in spherical coordinates is the essential mathematical basis for the present analysis. It is interesting to note that the efficiency for trapping does not reduce as rapidly as the reduction of the size of the trap. For a 100-fold reduction of the surface area of the trap, the average lifetime of the molecule in the trap-free region increases only about threefold. In other words, for a given total trap surface, a much more efficient trapping can be achieved by dispersing

TABLE I

EXAMPLES OF SURFACE TRAPS OF VARIOUS SIZES ON A SPHERICAL MEMBRANE*

Trap size			Average lifetime in diffusion space $\bar{\tau}$ (*min*)		
$1 + x_0$	θ_0	S_t	$D = 5.0 \times 10^{-9}$	$D = 1.0 \times 10^{-9}$	$D = 2.0 \times 10^{-10}$ cm^2/s
0.1	25.8°	$0.2\ \pi r_0^2$	16	76	4.0×10^2
0.01	8.1°	$0.02\ \pi r_0^2$	32	1.6×10^2	8.1×10^2
0.001	2.6°	$0.002\ \pi r_0^2$	49	2.5×10^2	1.2×10^3

*$x_0 = -\cos\theta_0$, $2\theta_0$ = convergence angle enclosing the trap, S_t is the surface area of the trap, and $\bar{\tau}$ is the mean lifetime of the molecule in the trap-free region of the membrane. Three different values of D of the molecules in the trap-free region of the membrane are used. The radius of the spherical membrane (r_0) is taken to be 15 μm.

the trap into many small traps on the surface. This is consistent with an intuitive argument that the latter provides larger boundary surface for trapping the molecules.

III. EXPERIMENTAL FINDINGS

Localization of Cell Surface Receptors Induced by Cell-Cell Contact

Reaggregating embryonic cells develop communication (gap) junctions when they come into contact with one another (19,20). Cell surface glycoproteins are believed to play an important role in the initial adhesion as well as the subsequent formation of gap junctions between these cells (21,22). Previous studies on the lectin receptors on the surface of embryonic *Xenopus* myotomal cells (23–25) have shown that many lectin receptors, presumably glycoproteins, are mobile in the plane of the plasma membrane. In the present study, we used fluorescence-labeled lectins, concanavalin A (Con A) and soybean agglutinin (SBA), to map the distribution of surface glycoproteins before and after the *Xenopus* myotomal cells were manipulated into contact. We found that a subpopulation of the SBA receptors were preferentially accumulated at the site of the contact within 10 min after the contact is made. This accumulation is a passive process independent of the cell metabolism, and is consistent with the notion that the contact site serves as a trap for some of the mobile SBA receptors. Quantitative analysis of this phenomenon with the model developed in the theory section will be presented in the next section.

Embryonic *Xenopus* myotomal cells were obtained by a procedure described in a previous report (23). The cells were grown in monolayer culture for two days before they were used in the experiments. Isolated, spherical myotomal cells (average diameter 30 ± 5 μm) were brought into contact by pushing one cell toward another with a glass micropipette that was manually controlled by a Leitz micromanipulator. The diameter of the area of contact between all the cell pairs produced by manipulation was approximately equal to the average radius of the cells. Typically, about 10–15 pairs can be produced within a 10-min period of manipulation. After the cells were brought into contact for a period of time, the cells were labeled with fluorescein-conjugated Con A (25 μg/ml) or SBA (100 μg/ml) for a period of 15–30 min at 4°C. The cells were then washed with saline and immediately fixed with cold acetone (at −4 to 0°C) after washing. Fixed cells were preserved in 95% glycerol before the microfluorimetric measurements were carried out.

The use of microfluorimetry in mapping the distribution of fluorescently labeled membrane receptors on isolated, single cells was described in a previous report (24). In the present study, the fluorescence intensity at the area of contact between the cells was sampled by placing an 8-μm measuring aperture directly at the middle of the contact area, and the intensity of the noncontact areas of both cells was sampled by placing the aperture at the region of the surface farthest away from the contact area (see also Fig. 6). Background fluorescence intensity at adjacent cellfree regions was subtracted from all intensity measurement on the cell. The corrected readings of the intensities were then used to calculate an accumulation index (A.I.) for the redistribution of the labeled receptors defined by the following formula:

$$\text{A.I.} = (I_c - 2I_{nc})/I_{nc} \tag{10}$$

where I_c and I_{nc} are the corrected fluorescence intensities at the contact and noncontact areas, respectively. I_{nc} is obtained by taking the average of intensities measured from both cells. A factor of 2 is used in the formula since the total membrane area at the contact side is twice that of the noncontact site due to the contribution from both cells of the cell pair. If no

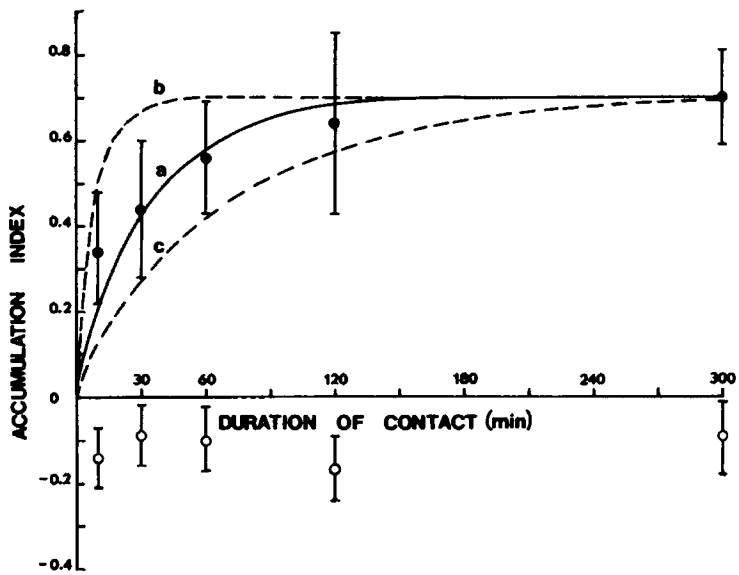


FIGURE 4 Accumulation of soybean agglutinin (SBA) receptors induced by cell-cell contact. 2-d-old sypherical *Xenopus* myotomal muscle cells were manipulated into contact for various periods of time. The cells were then incubated with fluorescein-labeled SBA (F-SBA) or Con A (F-Con A), fixed in cold acetone and preserved in 95% glycerol. Microfluorimetric measurements of the intensity of fluorescence ring staining at the contact and the noncontact regions of the cells were carried out (see text for details). Accumulation index is a normalized difference in fluorescence intensities at these two regions (see Eq. 10). Note that there is a rapid accumulation of SBA receptors within 10 min of cell-cell contact (●). Con A receptor distribution (○), on the other hand, showed no accumulation. The diameter of the contact area was set at approximately the average radius of the cells (15 μm). Each data point represents average accumulation index obtained from measurements on at least 14 cell pairs (see also Table II). Error bars represent standard errors. Solid curve is the prediction of surface trap theory using a diffusion coefficient of 2.5×10^{-9} cm^2/s (*a*) for the trappable SBA receptors. Dashed curves represent the limits for acceptable fit, using diffusion coefficients of 9.3×10^{-9} (*b*) and 1.2×10^{-9} cm^2/s (*c*).

redistribution is induced by the contact, the A.I. has a value of about 0. If an accumulation occurs after the contact is made such that the intensity at the contact area is three times that of the noncontact area, the A.I. has a value of about 1.

Fig. 4 shows the A.I. obtained when cells were in contact for periods of 10 ± 5, 30 ± 5, 60 ± 5, 120 ± 5, and 300 ± 5 min, and subsequently labeled with either fluorescent Con A or SBA. The rise of A.I. for the SBA receptors suggests a rapid accumulation of the SBA receptors within 10 min after cell-cell contact. The A.I. for the Con A receptors, on the other hand, had values slightly below zero for measurements done for all duration of contact, indicating that the Con A receptor did not accumulate at the contact site. The results are summarized in Table II. The average values of A.I. have relatively large uncertainty, this was due to the large variation in the extent of accumulation in various cell pairs produced in a given culture. As an example, in the experiment examining the postcontact labeling of F-SBA, 16 out of 22 cells showed positive accumulation indices after 10 min of cell-cell contact; and 29 out of 33 cells showed positive indices after 5 h of contact. The rest of the cells show either zero or negative indices. The tabulated values are averages including all the values collected in one or more cultures.

Binding of Con A and SBA to their receptors is known to impede the rate of lateral diffusion of these receptors (23,25). When the cells were preincubated with Con A (100 μg/ml) for 10 min before the cells were pushed into contact, no accumulation of the SBA receptors was observed by labeling the cells with fluorescent SBA after 1 or 2 h of contact.

TABLE II
REDISTRIBUTION OF MYOTOMAL MEMBRANE COMPONENTS INDUCED BY CELL-CELL CONTACT*

Treatment and the fluorescent label examined	Duration of cell-cell contact				
	10 min	30 min	1 h	2 h	5 h
Postcontact labeling with R-Con A	−0.14 ± 0.09 (30)	−0.08 ± 0.07 (24)	−0.10 ± 0.07 (34)	−0.17 ± 0.06 (45)	−0.09 ± 0.09 (31)
Postcontact labeling with F-SBA	0.34 ± 0.13 (22)	0.43 ± 0.15 (16)	0.54 ± 0.12 (31)	0.63 ± 0.21 (14)	0.69 ± 0.11 (33)
Precontact labeling with F-SBA	N.D.‖	N.D.	0.08 ± 0.14 (16)	0.07 ± 0.27 (6)	N.D.
Precontact incubation with Con A and postcontact labeling with F-SBA	N.D.	N.D.	0.07 ± 0.07	0.03 ± 0.09	N.D.
Postcontact incorporation of dil	N.D.	N.D.	−0.14 ± 0.05 (3)	−0.23 ± 0.17 (9)	N.D.
†Incubation with metabolic inhibitors and postcontact labeling with F-SBA	0.36 ± 0.12 (13)	0.27 ± 0.06 (34)	0.29 ± 0.08 (31)	N.D.	N.D.
§Incubation with cytochalasin B and colchicine, postfield labeling with F-SBA	N.D.	N.D.	1.04 ± 0.26 (18)	1.10 ± 0.18 (21)	N.D.

*The redistribution is determined quantitatively by measuring the difference of fluorescence intensities at the contact and the noncontact area of the cell, in terms of accumulation index defined by Eq. 10 (see text for details). Numbers in the parentheses refer to the number of cell pairs measured.
†The cells were incubated in 1 mM dinitrophenol and 10 mM sodium azide for at least 30 min before they were brought into contact. These inhibitors are also present during the period of manipulation and postcontact incubation.
§The same treatment as that described for metabolic inhibitors. Cytochalasin B: 10 μg/ml, and colchicine:20 μM.
‖N.D., no data.

Similarly, prelabeling of the cells with F-SBA before the contact prevented the accumulation of the SBA receptors induced by the contact.

Alteration of the surface morphology at the contact site, e.g. the folding or interdigitation of the membrane, could give rise to fluorescence intensity due to increased total membrane area. The relatively stronger labeling by SBA as compared to Con A could be accounted for if Con A molecules are less effective in penetrating the space between the contacting membrane, and the positive A.I. value for SBA labeling is simply the result of increased membrane area related to the alteration of surface morphology. To exclude this possibility, we did an additional experiment on the incorporation of an exogenous fluorescent lipid, 3,3'-dioctadecylindocarbocyanine iodine (dil). The cell pairs that were in contact for 1 or 2 h were incubated with saline containing 3 μg/ml of dil for 10 min, and the surface distribution of dil fluorescence was then measured microfluorimetrically to determine the A.I. of dil. As shown in Table II, the negative A.I. value for dil fluorescence argues against the increase of total surface area at the contact site, since the dil molecules, which are much smaller than both Con A and SBA, presumably were easily incorporated into the membrane at the contact area.

It may be noted that, for the same convergence angle, the surface area of the noncontact region is slightly greater than that of the contact region due to the absence of curvature in the latter. Assuming the contact area sustains a convergence angle of 1 rad (57.3°), the flattening of the cells at the contact region result in a 4.2% decrease in the total surface area. Thus, the accumulation index, as defined by Eq. 10, should be -0.08 if no accumulation occurs. This may partly account for the negative indices for Con A receptors and incorporated dil.

The accumulation of SBA receptors at the cell-cell contact site is apparently a passive process, requiring no metabolic energy supply by the cell. Preincubation of the cells with metabolic inhibitors, dinitrophenol (1 mM), sodium azide (1 mM) and sodium fluoride (10 mM), did not reduce the A.I., as shown in Table II. Cytoskeletal structures, e.g. microtubules and microfilaments, also do not seem to be involved in the induction of receptor accumulation we observed, since treatment with drugs (colchicine and cytochalasin B) that disrupt these structures in other cell types did not decrease the accumulation induced by cell-cell contact.

Finally, we demonstrated that the SBA receptors are indeed laterally mobile in the *Xenopus* myotomal cells of these 2-d cultures. The diffusion coefficient for these receptors was measured by the postfield relaxation method previously reported (24,25). Fig. 5 shows the decay of asymmetric SBA receptor distribution after the termination of an extracellular steady electric field of 10 V/cm, which had been applied to the isolated myotomal cells for 30 min. As shown previously (25), the diffusion coefficient of the molecules can be determined from the characteristic $1/e$ decay time (τ_d) of the postfield asymmetry index, i.e., $D = r_0^2/2\,\tau_d$, where r_0 is the radius of the cell. The best fit of the first-order exponential curve shown in Fig. 5 indicates that the electrophoretically mobile SBA receptor has an average diffusion coefficient of 1.9×10^{-9} cm^2/s, assuming the radius of the cells was 15 μm. Immobile receptors do not contribute to the decay of asymmetry index, hence are inherently not included in the above estimate of average diffusion coefficient. It may be noted that the fraction of SBA receptors that were accumulated to the contact site represents only a small fraction of the mobile SBA receptors (see next section), which are presumably a rather

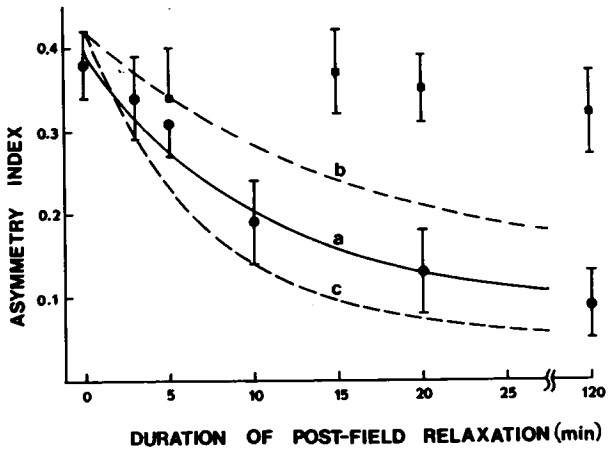


FIGURE 5 Lateral diffusion of myotomal surface SBA receptors. Accumulation of the SBA receptors on isolated spherical myotomal cells was first produced by exposure of the cells to a steady extracellular electric field of 10 V/cm for 30 min. The cells were then allowed to relax in culture for various periods of time before labeling of F-SBA was carried out. The asymmetry index was determined by measuring the differences in the intensity of fluorescence of the cathode and anode-facing poles of the cell, using a microfluorimeter (24). The decay of asymmetry index (●) indicates the back-diffusion of the unlabeled receptors after the termination of the field. No back-diffusion was observed when the cells were labeled with F-SBA immediately after the termination of the field (■). Each data point represents an average asymmetry index obtained from measurements on at least 30 cells. Error bars represents 95% confidence limits. The solid curve depicts the best fit by a exponential decay of asymmetry index for molecules possessing a diffusion coefficient (D) of 1.9×10^{-9} cm^2/s (a) (23). Dashed curves represent the limits of acceptable theoretical fits, with D values of 1.2 (b) and 2.7×10^{-9} cm^2/s (c).

heterogenous group of glycoproteins on the cell surface. The fractional amount of electrophoretically mobile receptor can be determined by the following analysis.

Assuming all the SBA receptors that remain at the anodal pole of the cells are immobile in the present electrophoresis experiments (10 V/cm field applied for 30 min), the relative angular distribution of F-SBA fluorescence is given by (see also reference 24):

$$I(\theta) = 55 \exp[-0.96(1 + \cos\theta)] + 45$$

where the relative intensities of fluorescence at 0° and 180° are set to be 45 and 100, respectively (asymmetry index 0.38). Integration of the fraction of redistributed fluorescence indicates that the electrophoretically mobile SBA receptors are at least 26% of the total SBA receptors on the cell surface.

In conclusion, we have shown that after the *Xenopus* myotomal cells were manipulated into contact, some of the surface SBA receptors rapidly accumulate toward the site of contact. This accumulation of the SBA receptors is unlikely to result from an alteration in membrane morphology at the contact site, and appears to be a result of passive lateral diffusion of the SBA receptor into a trap formed at the site of cell-cell contact.

IV. CELL-CELL CONTACT SITE AS A TRAP FOR SURFACE GLYCOPROTEINS

In this section, we use our surface trap theory to analyze the experimental findings described in the previous section. We will demonstrate that the accumulation of myotomal surface SBA receptors induced by cell-cell contact in myotomal culture can be accounted for by assuming the contact site serves as a trap for a subpopulation of the mobile fraction of SBA receptors.

As shown in Fig. 6, we assume that immediately after the myotomal cell pair is manipulated into contact, the contact area (S_t) becomes a trap for some of the diffusing SBA receptor molecules in the noncontact area (S_0). In the nomenclature of the theory section, we have the following relation:

$$S_t = 4\pi r_0^2 - S_0 = 2\pi r_0^2(1 + x_0)$$

Where r_0 is the radius of the cell, $x_0 = -\cos\theta_0$, and $2\theta_0$ = convergence angle enclosing the trap. From Eq. 8, the total number of molecules that have diffused into the contact area and have been trapped at time t is given by

$$P(0) - P(t) = P(0)\left[1 - \sum_{s=0}^{\infty} B_s \exp(-t/\tau_s)\right]$$

and the increase in the surface density of the receptor molecules $C_T(t)$ in the trap area is given by

$$C_T(t) = \frac{P(0) - P(t)}{S_t} = \frac{(1 - x_0)C_0}{1 + x_0}\left[1 - \sum_{s=0}^{\infty} B_s \exp(-t/\tau_s)\right].$$

Assuming the fluorescence intensity is linearly proportional to the surface density of the receptor molecules, the intensity measured at the contact (I_c) and noncontact areas (I_{nc}) of the cell can be expressed by

$$\begin{aligned} I_c(t) &= 2\alpha[C_0 + C_B + C_T(t)] \\ I_{nc}(t) &= \alpha[C_B + C(1, t)] \end{aligned} \tag{11}$$

where α is a constant coefficient, C_0 is the initial uniform density of trappable SBA receptors before the contact is made, and C_B is the density of the immobile fraction of the SBA receptor

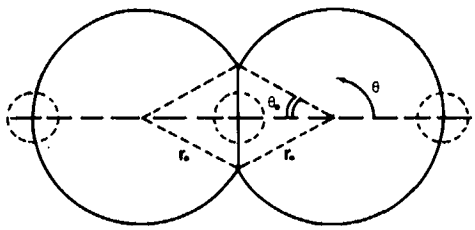


FIGURE 6 Schematic model of cell-cell contact. The cross section of one cell pair shows the diameter of the contact area is equal to about the radius of the cell, the convergence angle enclosing the contact area is 2 θ_0, and the contact area is assumed to be a perfect trap for soybean agglutinin receptor molecules diffusing in the plane of the membrane. Dashed circles indicate the positions of microfluorimetric measurement of fluorescence intensity.

molecules; $C(1, t) = C(\theta = 0, t)$ is the density of the molecules at the noncontact area $\theta = 0$ and time t, where $C(1,0) = C_0$. A factor of 2 is introduced for $I_c(t)$ because of the contribution of the trapped molecules by two contacting cells.

Our experimental studies use an accumulation index (A.I.) to compare the relative fluorescence intensities (I_c and I_{nc}) of SBA receptor labeling between the contact area and noncontact area, as defined by Eq. 10. From Eqs. 7–11, and $P_{\nu_s}(1) = 1$, we obtain the accumulation index

$$\text{A.I.} = \frac{\left\{\dfrac{4}{1+x_0} - \sum_{s=0}^{\infty}\left[\dfrac{I_s(x_0)}{H_s(x_0)} + \dfrac{1-x_0}{1+x_0}B_s\right] 2\exp(-t/\tau_s)\right\}}{\left\{\dfrac{C_B}{C_0} + \sum_{s=0}^{\infty}\dfrac{I_s(x_0)}{H_s(x_0)}\exp(-t/\tau_s)\right\}}. \quad (12)$$

When $t \gg \tau_0$, A.I. approaches a constant value given by $4C_0/(1 + x_0)C_B$. The contact areas produced in these experiments were about 1 rad, i.e. $2\theta_0 = 1$ rad and $1 + x_0 = 0.12$. Fig. 4 indicates, when $t \gg \tau_0$, A.I. $\simeq 0.75$. Thus, we obtain a ratio of C_0 to C_B to be about 0.02 (or $C_B/C_0 = 47.6$). This low C_0 to C_B ratio indicates that the fraction of SBA receptor molecules trapped at the contact site is only about 2% of the total SBA receptors in the membrane. Since at least 26% of the SBA receptors in the membrane are electrophoretic mobile (see previous section), these accumulated SBA receptors represent only a small fraction of the mobile SBA receptors in the muscle membrane.

For the time-course of accumulation of SBA receptors shown in Fig. 3, we use the first order approximation of Eq. 12, which is given by

$$\text{A.I.} = \frac{\left\{\dfrac{4}{1+x_0} - \dfrac{I_0(x_0)}{H_0(x_0)}\left[1 + \dfrac{I_0(x_0)}{1+x_0}\right] 2\exp\left[-\dfrac{D}{r_0^2}\nu_0(\nu_0+1)t\right]\right\}}{\left\{\dfrac{C_B}{C_0} + \dfrac{I_0(x_0)}{H_0(x_0)}\exp\left[-\dfrac{D}{r_0^2}\nu_0(\nu_0+1)t\right]\right\}}. \quad (13)$$

Where $1 + x_0 = 0.12$ for 1 rad contact; $\nu_0 = 0.33$, $H_0(x_0) = 0.96$, and $I_0(x_0)/H_0(x_0) = 1.32$, as shown by the graphs in reference 14; $C_B/C_0 = 47.6$, obtained by the plateau value of A.I. Eq. 13 predicts the solid curve shown in Fig. 4 for cells with radius 15 μm, and a diffusion coefficient (D) of the trappable SBA receptors of 2.5×10^{-9} cm^2/s. This D value is close to the average value determined independently by the postfield back diffusion experiments on the electrophoretically mobile receptors (range: 1.2–2.7 $\times 10^{-9}$ cm^2/s), as shown in Fig. 5.

Finally, we note that the present analysis is based on the simplest assumption that the contact area serves as a perfect trap. For an imperfect trap where trappable molecules may escape, the diffusion coefficient inferred from other analysis on the SBA receptor accumulation becomes an underestimate.

V. DISCUSSION

The present study focuses on a passive diffusion mechanism by which mobile membrane components become localized at a specific region of the cell membrane due to the presence of a local surface trap. In principle, many types of surface modulation could provide a molecular basis for an efficient surface trap for diffusing molecules. At the site of cell-cell contact,

molecules on the surface of each cell may provide specific ligands for molecules on the other cell, and the resulting formation of immobile intercellular linkages can thus account for trapping the linked molecules. Enzyme-substrate interactions between the adjacent cells, especially that of the surface glycosyltransferases activity, have been postulated for a molecular basis of cell-cell adhesive interactions (22). Modulation of membrane components by the binding of exogenous ligands may also alter the balances of repulsive and attractive forces between the membrane components in such a way that favors the formation of immobile molecular aggregates (12). The effective presence of the trophic substances (6) that produced the latter effect could be restricted to, for example, the cell-cell contact region, and lead to a localized aggregation (trapping) of membrane components.

Bretscher (26) has recently pointed out that the knowledge about exactly how fast proteins diffuse laterally in the membrane is crucial for understanding many of their complex cellular functions. The present study provides another example. As shown in Table I, the efficiency of surface trap degrades rapidly as the diffusion coefficient is reduced. In the case of the accumulation of SBA receptors induced by cell contact, the diffusion-trap model can be a plausible mechanism only if the diffusion coefficient of the receptor is within the range of $1.2–9.3 \times 10^{-9}$ cm^2/s. The average diffusion coefficient of the electrophoretically mobile SBA receptors found by the postfield relaxation method, i.e. 1.9×10^{-9} cm^2/s, is close to the lower limit of this range. We consider that this result supports the diffusion-trap model satisfactorily, since the accumulated SBA receptors consist of only a small fraction of the mobile SBA receptors on the surface; their diffusion coefficient could be much higher than the average value obtained from the postfield relaxation method.

2-d-old cultured *Xenopus* myotomal cells become electrically coupled within 30 min after they are manipulated into contact (Poo, unpublished observation). The rapidity of the accumulation of some of the SBA receptors at the contact site is sufficient to qualify these receptors to be a candidate for the gap-junction precursor. Presumably, a proteolytic cleavage of the precursor molecules is required before a permeable channel between neighboring membrane is formed (21). Alternatively, the accumulation of the SBA receptors we observed may merely serve an adhesive function between the cells.

In summary, the present study illustrates a quantitative method for analyzing molecular redistribution on the surface of a spherical cell. It demonstrates that the localization of cell surface receptors induced by cell-cell contact can be accounted for by assuming the contact site serves as a trap for a specific population of the diffusing receptors. The theory may also prove useful in determining quantitatively whether other cellular processes, such as the localization of neurotransmitter receptor toward the site of nerve contact (1, 2) and the localization of hormone-receptor complexes toward the "coated pits" on the cell surface (7), also operate by a similar passive mechanism.

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