Redistribution of Cell Surface Receptors Induced by Cell–Cell Contact

IDA CHOW and MU-MING POO
Department of Physiology and Biophysics, University of California, Irvine, California 92717

ABSTRACT   Cell surface lectin receptors underwent rapid redistribution after embryonic *Xenopus* myotomal muscle cells were manipulated into contact in culture. Soybean agglutinin (SBA) receptors became highly concentrated at the contact area and concanavalin A (Con A) and ricin receptors were depleted at the same region. The accumulation of SBA receptors was greatly reduced by the presence of SBA specific sugars in the incubating medium, by precontact binding of SBA to the surface and by lowering the temperature, but it was unaffected by prolonged treatments with metabolic inhibitors. It is culture-age dependent: older cultures showed a markedly reduced extent of accumulation, and the high accumulation resulting from contact made in younger cultures disappeared with time in culture. These findings are consistent with the notion that specific molecular interaction between the contacting surfaces results in a redistribution of preexisting rapidly diffusing surface receptors. In support of this notion, ligand-free SBA and Con A receptors were shown to be laterally mobile in the membrane, and at least a subpopulation of the SBA receptors contains physically distinct molecules from the Con A receptors. We suggest that such contact-induced redistribution of various surface components may play a role in the interaction between embryonic cells.

Cell surface components play an important role in cell-cell recognition, intercellular communication, and cellular differentiation during embryonic development (for reviews, see 2, 7, 9-11, 19). Specific interaction between molecules on the surfaces of contacting cells within the tissue seems to be an essential step for many of these developmental processes. Previous studies of selective cell-cell adhesion have clearly implicated the involvement of specific cell surface glycoproteins (8, 11, 20, 21). During the past decade, it has also become quite clear that the physical state of cell surface glycoproteins is highly dynamic. Many glycoproteins, especially those in the embryonic plasma membrane, are capable of rapid lateral migration in the plane of the membrane (5, 12, 16, 17). Two questions immediately arise: does lateral migration of membrane components play any role in establishing specific cell-cell interaction; or conversely, can specific intercellular interaction be responsible for inducing lateral rearrangement of membrane components into specific patterns of membrane topography that is characteristic of many differentiated cells?

As a first step in studying these problems, we examined the distribution of plasma membrane glycoproteins on cultured embryonic muscle before and after the cells were manipulated into contact. Preliminary findings indicated that many surface soybean agglutinin receptors, presumably glycoproteins containing D-galactose and/or N-acetyl-α-D-galactosamine resi-

dues, are induced to accumulate at the site of cell-cell contact. The rate of this accumulation of surface receptors is consistent with the idea that the contact site serves as a trap for rapidly diffusing molecules in the plane of the cell membrane (3). In our study, we examined in detail this contact-induced redistribution of cell surface components. The results indicate that the contact-induced accumulation involves specific sugar residues on surface receptors, it results from lateral redistribution of preexisting surface receptors, and it is a phenomenon dependent upon culture age. We suggest that this contact-induced redistribution of surface receptors may play an important role in specific cell-cell interaction during development. In particular, it serves to enhance specific cell-cell adhesion during the initial contact between embryonic cells. Part of the results were reported previously in a brief form (4).

MATERIALS AND METHODS

Cell Culture and Manipulation of the Cells into Contact

Myotomal cells of *Xenopus laevis* embryos at stages 17-19 (13) were dissociated and cultured on clean glass microscope slides following the procedure previously described (18). Briefly, neural tube and underlying mesodermal tissue were dissected from the embryo and allowed to dissociate in Ca²⁺-Mg²⁺-free solution (58.2 mM NaCl, 0.7 mM KCl, 0.3 mM EDTA, penicillin-Streptomycin-250 UI, pH 7.8). The dissociated myotomal cells (mainly muscle cells) were then plated

on slides in culture medium containing 10% Leibovitz medium (Gibco Laboratories, Grand Island Biological Co., Grand Island, NY), 5% fetal bovine serum (Gibco Laboratories) and 85% Steinberg's solution. The latter consists of 58.2 mM NaCl, 0.7 mM KCl, 0.4 mM $Ca(NO_3)_2 \cdot H_2O$, 1.3 mM $MgSO_4 \cdot 2H_2O$, 4.6 mM Tris, and 250 UI penicillin-Streptomycin. The pH of the culture medium was 7.8. After one day in culture, most of the dissociated embryonic muscle cells (both spindle-shaped and spherical) were found to adhere to the glass substratum. They can be easily identified morphologically by their size and shape, and physiologically by their acetylcholine sensitivity and contractile response (see references 14, 16, 18).

Isolated, spherical embryonic muscle cells in culture of various ages were brought into contact by detaching one cell from the substratum with a micromanipulator-controlled glass micropipette and pushing the detached cell to make contact with another isolated muscle cell still firmly attached onto the glass surface. The area of cell-cell contact produced usually had a diameter of about the radius of the cell ($\sim$15 μm).

Fluorescence Labeling

Various fluorescently labeled lectins were used to map the distribution of specific sugar-containing surface components of the cultured muscle cells: soybean agglutinin (SBA), wheat germ agglutinin (WGA), concanavalin A (Con A), Ricinus communis agglutinin (RCA_{120}), Dolichos biflorus agglutinin (DBA), peanut agglutinin (PNA), and Ulex europeus agglutinin (UEA I). All lectins were purified and conjugated with fluorescein isothiocyanate by Vector Laboratories (Burlingame, California 94010). In some experiments, tetramethylrhodamine-conjugated Con A from the same source was also used. Labeling solution was Steinberg's saline containing 25–250 μg/ml of the fluorescent lectin. Both labeling and washing procedures were done at low temperature (0°–4°C). Microfluorimetric measurements were performed on living cells immediately after fluorescence labeling. In these cultured embryonic muscle cells, no lectin-induced capping of receptors was observed. At room temperature (22°C), the internalization of lectin-receptor complexes was also insignificant during the first hour (the interval for most fluorimetric measurements) following labeling, as evidenced by the absence of cytoplasmic staining and clear ring staining around the perimeter of the cell. "Patching" of lectin-receptor complexes on the cell surface, however, was frequently observed following the labeling (see also Fig. 3). This is presumably due to the aggregate formation induced by multivalent lectin (12).

Microfluorimetry

Fluorescence intensities at the cell-cell contact and at noncontact areas diametrically opposite to the contact site of both cells of the cell pair were measured microfluorimetrically according to a method described previously (17). Briefly, a measuring aperature of 8 μm in diameter was focused on the perimeter of the cell, and the intensity of the "ring" staining was measured by a Zeiss microphotometer (PM1) through the aperature. Background fluorescence intensity was taken at adjacent cell-free regions and subtracted from the readings taken from the cell-pairs to yield corrected fluorescence intensity. Accumulation of lectin receptors at the cell-cell contact area was determined by an accumulation index (AcI) defined by the following formula: $AcI = (I_c - 2I_{nc})/I_{nc}$, where I_c is the corrected fluorescence intensity at the contact area, and I_{nc} is the average corrected intensity at the noncontact areas from both cells. A factor of 2 is used in the formula because both cells' membranes contribute to the total membrane area at the contact site. 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 AcI has a value of $\sim$1. When no accumulation occurs, it has a value of -0.08. This slightly negative value is due to the fact that there is usually a flattening of the cell surface at the site of contact, resulting in a 4% decrease in the total surface area as compared to the noncontact region (see also reference 3). AcI values more negative than -0.08 indicate depletion of the receptors from the contact area. All the artificially produced cell pairs that remained in contact after staining and washing procedure were measured. The AcI value from a particular experiment refers to the average of all AcI values of individual cell pairs from one or more cultures.

RESULTS

Redistribution of Lectin Receptors Induced by Cell-Cell Contact

In the first series of experiments, spherical muscle cells in 1-d-old Xenopus cultures were manipulated into contact. After 30 min of contact, the cell surface was labeled with fluorescein-conjugated lectins. The intensities of fluorescence at the cell-cell contact area and at the noncontact area were measured

microfluorimetrically to determine the accumulation index (AcI). Table I gives AcI for different lectin receptors after a 30-min cell-cell contact period. Of the six types of lectin receptors examined, only SBA receptors showed strong accumulation at the contact site. DBA, WGA, and PNA receptors exhibited only slight accumulation at the contact site, whereas receptors for Con A and RCA_{120} seemed to be depleted at the contact site (AcI values < -0.08, see Methods).

Fig. 1 depicts photomicrographs of representative cells from this study. SBA receptors showed marked accumulation at the contact area (Fig. 1 b). Accumulation of WGA receptors was less intense (Fig. 1 c), and Con A receptors did not accumulate at the contact area (Fig. 1 d). The binding of fluorescent SBA was specific, because the fluorescence staining was greatly reduced when their specific sugars were present in the incubating medium (see also Fig. 3). For example, in the presence of 10 mM D-galactose, the same labeling procedure resulted in a drop of absolute fluorescence ring staining of F-SBA in arbitrary units from 8.79 ± 0.37 (N = 50, ± SE) to 0.81 ± 0.08 (N = 80, ± SE), as measured microfluorimetrically. There appears to be no significant morphological alteration (e.g., folding) of plasma membrane at the site of contact. When fluorescent lipid 3,3'-dioctadecylindocarbocyanine iodine (diI) was incorporated into the membrane after 30 min of cell-cell contact (10 μg/ml, 3-min incubation), presumably uniformly throughout all membrane area, no accumulation of fluorescence was found at the cell-cell contact area (Fig. 1f). The AcI was -0.09 ± 0.08 (N = 33, ± SE), a value very close to the theoretical value (-0.08) predicted for membrane components showing no accumulation (see Methods).

The time course of receptor accumulation was determined by measuring the AcI after different contact periods. Results for SBA, WGA, and Con A receptors are presented in Fig. 2. 10 min after contact, significant accumulation was found for SBA receptors and WGA receptors at the contact site. With time, more SBA receptors accumulated at the contact site and

TABLE I

Distribution of Lectin Receptors in Embryonic Muscle Cell Membrane Induced by Cell-Cell Contact

Fluorescent lectin used*	Specific sugars	Accumulation index ± SEM after 30 min of contact‡
Soybean agglutinin	α or β-D-galactose N-acetyl-α-D-galactosamine	2.65 ± 0.20 (99)
Peanut agglutinin	β-D-galactose N-acetyl-β-D-galactosamine	0.67 ± 0.28 (15)
Dolichos biflorus agglutinin	N-acetyl-α-D-galactosamine	0.59 ± 0.16 (27)
Wheat germ agglutinin	N-acetyl-β-D-glucosamine N-acetyl-neuraminic acid	0.52 ± 0.15 (15)
Concanavalin A	α-D-glucose α-D-mannose	-0.30 ± 0.07 (55)
Ricinus communis agglutinin$_{120}$	β-D-galactose	-0.48 ± 0.06 (35)

* Lectins conjugated with fluorescein isothiocyanate were used to label the cell surface after 30 min of cell-cell contact. Labeling was carried out in cold temperature (0°–4°C) for 30 min (except for PNA which was 2 h). The concentration of the lectin in the labeling solution used was within the range of 25–250 μg/ml.

‡ Accumulation index as defined in Materials and Methods. Numbers in parentheses indicate the number of cell pairs measured for each average.
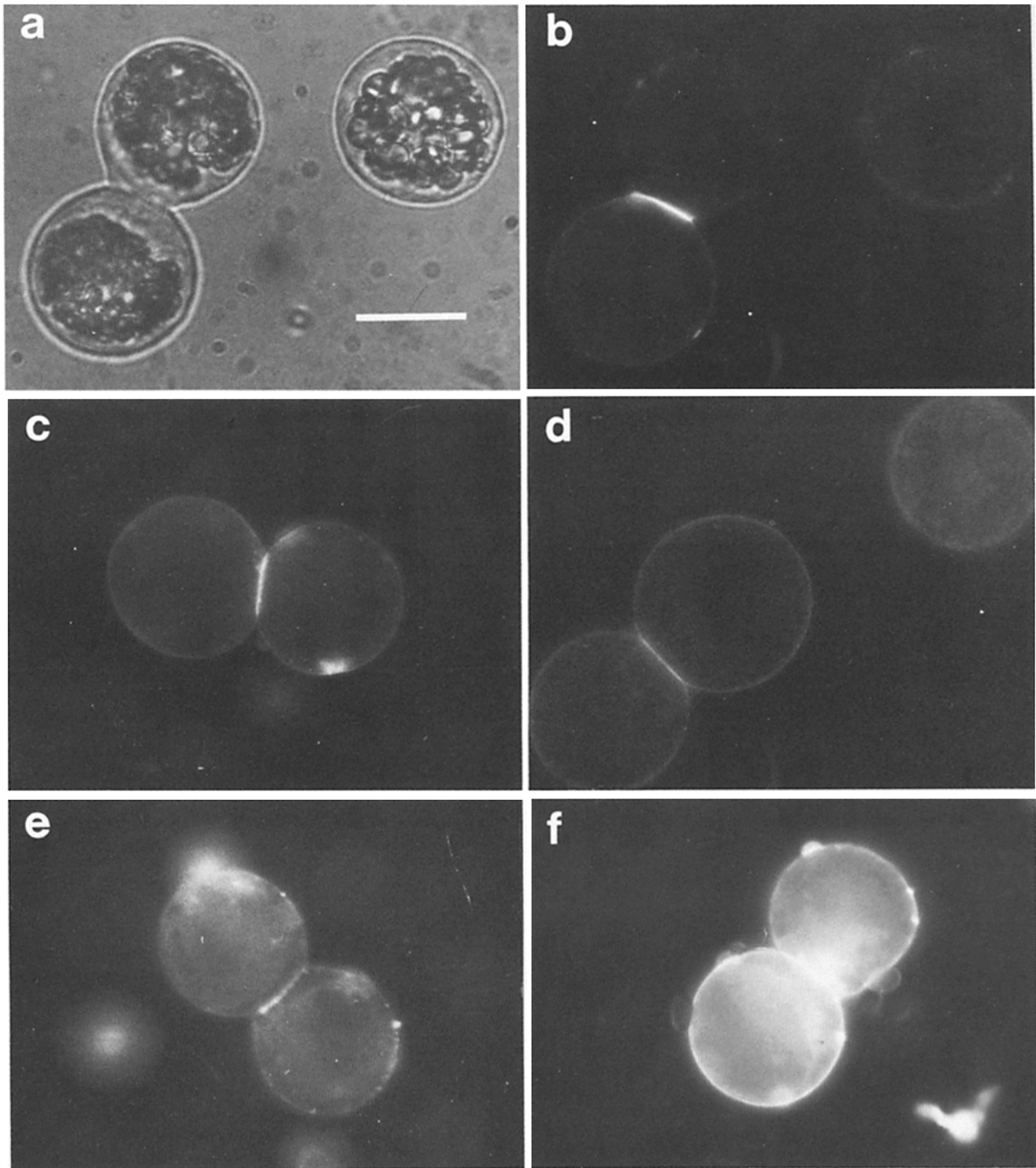
FIGURE 1 Representative photomicrographs of 1-d-old *Xenopus* muscle cells manipulated into contact for 30 min. Bright-field micrograph (*a*) and fluorescence micrograph (*b*) of a cell pair and an adjacent isolated cell labeled with F-SBA after the 30-min contact period, showing extensive accumulation of SBA receptors at the cell–cell contact site. The accumulation of WGA receptors at the contact site was less intense (*c*), as shown by F-WGA labeling of another cell pair after the 30-min contact period. No accumulation was seen for Con A receptors, as shown by F-Con A labeling (*d*), for cell prelabeled with F-SBA before contact was made (*e*), nor for fluorescent lipid (di-I) incorporated into the membrane after the 30-min contact period (*f*). The extent of accumulation was determined microfluorimetrically on a large number of cells (see Table I, II, and text). It may be noted that because of the nonlinearity of response in photographic films and prints, the visual impression from fluorescence photographs is unreliable. For example, direct measurements indicated that the higher intensity of F-Con A at the contact site (e.g., shown in *d*) did not exceed twice the intensity of staining at the noncontact area. Bar, 20 μm. × 1,000.
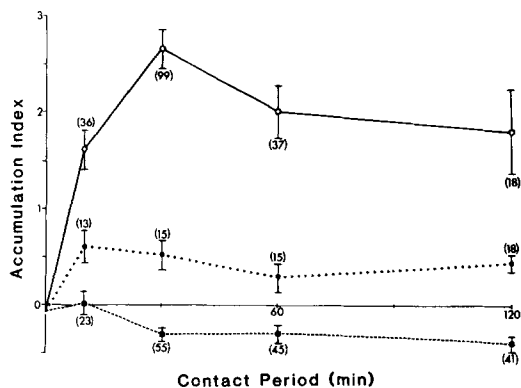
FIGURE 2 Accumulation indices of three types of lectin receptors after different cell-cell contact periods. SBA receptors (O) accumulated rapidly and the accumulation remained at high levels for at least 2 h after the contact. WGA receptors (●····●) also showed slight accumulation, whereas Con A receptors (■----■) showed depletion at the contact site (AcI <−0.08). At time zero, the AcI has has a theoretical value of −0.08, predicted for membrane components showing no accumulation (see Materials and Methods). Numbers in parentheses indicate the total number of cell pairs measured for each time interval. Error bars represent SEM.

such accumulation remained high for at least the first 2 h of contact. WGA receptors remained at about the same level from 10 min to 2 h of contact. Con A receptors, on the other hand, showed no accumulation for all contact durations studied. Because the AcI for Con A receptors was found to be significantly more negative than the theoretical value for the case of no accumulation (−0.08), it appeared that they were excluded from the contact area. This notion was supported by the finding that when cells were prelabeled with F-Con A, and then pushed into contact for 30 min, the AcI value was −0.07 ± 0.04 (N = 33, ± SE), a value close to that for membrane components showing no accumulation. Binding of Con A to the muscle cell surface is known to prevent lateral migration of the Con A receptors, presumably through the cross-linking action of the tetrameric Con A molecules (16, 18). Prelabeling with Con A may therefore have prevented migration of Con A receptors away from the contact site.

Accumulation of SBA receptors at the cell-cell contact area could be due to a contact-induced redistribution of preexisting SBA receptors on the cell surface or to selective local incorporation of new SBA receptors at the site of contact. However, concurrent with the increase of SBA receptor density at the contact site, there was a drop in the SBA receptor density at the noncontact area. The absolute fluorescence intensity of the noncontact areas measured in arbitary units was 3.62 ± 0.15 (N = 88, ± SE). This was 18% lower than that of the adjacent isolated spherical muscle cells in the same cultures: 4.38 ± 0.13 (N = 99, ± SE). Because the contact area occupied about 5% of the total cell surface (3), this 18% reduction was enough to account for the nearly three-fold increase in the intensity at the contact site. Although we have not excluded the possibility of a selective local incorporation at the contact site together with a degradation of receptors at noncontact area, our results are consistent with the simplest hypothesis that the accumulation results from a redistribution of preexisting receptors.

Changes in SBA Receptor Accumulation with Culture Age

In this study, the accumulation of SBA receptors induced by

cell-cell contact in 1-d-old cells was found to be much higher than that previously observed in 2-d-old cells from the same animal (3). This prompted us to further examine the age-dependency of this contact-induced accumulation. We found there is a sharp decline in the extent of accumulation during the first few days of culture. The accumulation index for SBA receptor induced by 30 min of contact was found to decrease from 2.65 ± 0.20 (N = 99, ± SE) in 1-d culture to 1.11 ± 0.24 (N = 27, ± SE) in 2-d culture, and 0.14 ± 0.08 (N = 35, ± SE) in 5-d culture. This reduction in accumulation index reflects not a reduced rate of accumulation, but the extent of accumulation. Even after 60 min of contact, 5-d-old cells showed an average index of 0.21 ± 0.12 (N = 20, ± SE), whereas for 1-d-old cells, it was 2.00 ± 0.27 (N = 37, ± SE). It was also noted that concomitant with this decrease in the contact-induced SBA receptor accumulation, the adhesiveness between the muscle cells also declined. In older cultures, cell pairs produced by micromanipulation frequently came apart during the labeling and washing procedures.

Interestingly, the accumulation of SBA receptors at cell-cell contact sites was found to disappear with time in culture. In a series of experiments, we manipulated 1-d-old cells into contact, and the labeling of F-SBA was delayed for prolonged periods. The resultant distribution of surface SBA staining showed progressively decreased accumulation at the contact site, and after a 24-h contact period, the cell pairs exhibited insignificant accumulation (AcI = 0.27 ± 0.11, N = 23, ± SE). These lower values of AcI could be accounted for by either selective removal of SBA receptors at the contact site or preferential insertion of additional SBA receptors at the noncontacted regions. We also noticed that cells that were in contact for prolonged periods adhered tightly to each other. Their separation by micromanipulation was much more difficult than those in contact for brief periods.

Specificity of Molecular Interaction at the Cell-Cell Contact Area

What kind of molecular mechanism is responsible for the accumulation of surface receptors at the contact site? The simplest hypothesis is that laterally mobile surface receptors are able to bind specifically to structurally complementary molecules on the other cell's surface and hence are trapped at the contact site. For example, binding of SBA-like surface molecules to specific sugar residues on the SBA receptors of the other cell surface could trap the diffusing SBA receptors at the contact site. The possibility of this type of lectin-sugar interaction was tested in the following experiments.

First, the cells were manipulated into contact for 30 min in the presence of high concentration (10 mM) of SBA-specific sugar, D-galactose. The cells were then washed thoroughly with fresh saline and labeled with F-SBA (all at 4°C). Subsequent examination of SBA receptor distribution showed a reduced accumulation, as compared to the accumulation of SBA receptor in the absence of the sugar or in the presence of another sugar (D-glucose) not specific to SBA (see Table II and Fig. 3). This result suggests cell surface D-galactose residues are involved in at least part of the molecular interaction responsible for the accumulation of SBA receptors.

In a second series of experiments, cells were preincubated with F-SBA and then manipulated into contact for 30 min. The postcontact examination showed insignificant accumulation of the labeled receptors (Table II and Fig. 1 e). Cell pairs were also manipulated into contact for 30 min in the continuous

TABLE II

Redistribution of Soybean Agglutinin Receptors in Embryonic Muscle Cell Membrane Induced by Cell-Cell Contact

Treatment	Accumulation index ± SEM after 30 min of contact
Control (contact at 22°C)	2.65 ± 0.20 (99)*
Contact at 15°C	1.92 ± 0.33 (26)
Contact at 8°C	0.87 ± 0.33 (22)
Precontact and contact incubation with galactose (10 mM), wash and F-SBA binding	1.40 ± 0.24 (48)
Precontact and contact incubation with glucose (10 mM), wash and F-SBA binding	2.20 ± 0.23 (25)
Precontact and contact incubation with galactose (10 mM) and NAcGal (10 mM), wash and F-SBA binding	1.18 ± 0.18 (28)
Precontact and contact incubation with SBA (100 μg/ml), wash and F-SBA binding	0.37 ± 0.13 (45)
Precontact incubation (30 min) with F-SBA (100 μg/ml)	0.28 ± 0.11 (21)
Precontact incubation (2 h) with metabolic inhibitors (NaN$_3$ 10^{-3} M, NaF 10^{-3} M and DNP 10^{-3} M)	2.94 ± 0.56 (24)
Precontact incubation (1 h) with colchicine (20 μM)	3.25 ± 0.50 (21)
Precontact incubation (1 h) with cytochalasin B (10 μg/ml)	0.84 ± 0.12 (30)
Precontact incubation (1 h) with colchicine and cytochalasin B	1.39 ± 0.18 (30)

* Numbers in parentheses indicate the number of cell pairs measured for each average.

presence of unlabeled SBA and postcontact labeled with F-SBA for examination of the SBA receptor distribution. Again, no significant accumulation was observed (Table II). These results can be accounted for by either one or both of the following possibilities: SBA binding to their surface receptors may block the recognition and binding of these receptors with the specific endogenous ligands on the opposing cell surface, thus preventing the entrapment of these receptors at the contact site. Alternatively, binding of SBA to their receptors may greatly impede the lateral diffusion of these receptors toward the contact site. Although the latter possibility was supported by the result on the lateral immobility of lectin-receptor complexes described in a later section, the former possibility has not been excluded.

To further test the notion that contact-induced accumulation of SBA receptors resulted from specific molecular interaction between the embryonic muscle cells, we examined the effect of contact with polystyrene beads and with dermatomal cells from *Xenopus* embryos. When inert beads were used to make contact with isolated spherical muscle cells for 30 min, no significant accumulation of SBA receptors was found at the contact site. This was true for both negatively charged polystyrene beads (Polysciences, Inc., Warrington, PA; 26 ± 10 μm in diameter), and positively charged polystyrene beads bearing surface hydrazide moieties (The Dow Chemical Co., Indianapolis, IN; 10 ± 5 μm in diameter). It was also found that neither type of bead adhered well to the cells, and many of them detached from the cells throughout the staining and washing processes. When isolated dermatomal cells were pushed into contact with isolated muscle cells, only a slight accumulation of fluorescent stain was observed (Fig. 3 *e* and *f*). Similarly, the adhesion between these two cell types was weak and many of the cell

pairs came apart throughout the experiments. No quantitative determination of AcI values was done in these experiments because F-SBA did not bind to the beads or to most of the dermatomal cells. These experiments using beads and dermatomal cells suggest that the extensive accumulation of SBA receptors between contacting muscle cells was not due to simple nonspecific surface events during cell-cell contact.

Effect of Lowering Temperature and Pharmacological Treatments

TEMPERATURE: Lowering the temperature during cell-cell contact periods reduced the extent of accumulation of SBA receptors at the contact site. At 8°C and 15°C, the accumulation of SBA receptors after 30 min of contact dropped to about 32% and 72% of the control value (at 22°C), respectively (Table II). At the lower temperatures, adhesion between the cells in contact became weaker than at 22°C, as suggested by the fact that fewer cell pairs remained adhered to each other after staining and washing procedure.

METABOLIC INHIBITION: The cells were incubated with Steinberg's saline containing sodium azide (10^{-2} M), sodium fluoride (10^{-3} M) and dinitrophenol (10^{-3} M) for 2 h before a 30-min contact period. No significant change in the accumulation of SBA receptors was found (Table II).The accumulation of SBA receptors induced by cell-cell contact thus appeared to be a process requiring limited or no metabolic energy supply. In these and following experiments using drug treatment, the cells were thoroughly washed with fresh medium before they were pushed into contact. The drug effects are presumably not a result of direct interference of the drugs with the contacting surfaces.

EFFECTS OF COLCHICINE AND CYTOCHALASIN B: When cells were pretreated with 20 μM colchicine (Sigma Chemical Co.) for 1 h before being pushed into contact, a slight increase in the AcI value was observed (Table II). In contrast, when cells were preincubated with cytochalasin B (10 μg/ml in 0.1% DMSO, Sigma Chemical Co.) for 1 h, the AcI dropped to 29% of the control value (Table II). Simultaneous pretreatment with colchicine and cytochalasin B (1 h) induced a lesser decrease in the AcI, 48% of the control value, than that caused by cytochalasin B alone (Table II).

Lateral Diffusion of SBA Receptors and SBA-Receptor Complexes

The above findings are consistent with the hypothesis that some of the cell surface SBA receptors are laterally mobile in the plane of plasma membrane, and that specific cell-cell contact induces the accumulation of SBA receptors through local trapping of diffusing SBA receptors in the membrane. To test this notion, lateral mobility of SBA receptors was studied on isolated, spherical muscle cells.

A steady electric field (10 V/cm) was applied to the culture for a brief period (10 min), and the distribution of surface receptors on isolated spherical muscle cells was examined by postfield labeling of cells with fluorescein-conjugated SBA. Quantitative measurement of the field-induced asymmetry in the distribution of SBA receptors was performed on large numbers of cells using a microfluorimetric method (17). Data were expressed in terms of the asymmetry index, which is defined as the normalized difference of fluorescence intensities on opposite poles (cathode or anode oriented) of the cell (see
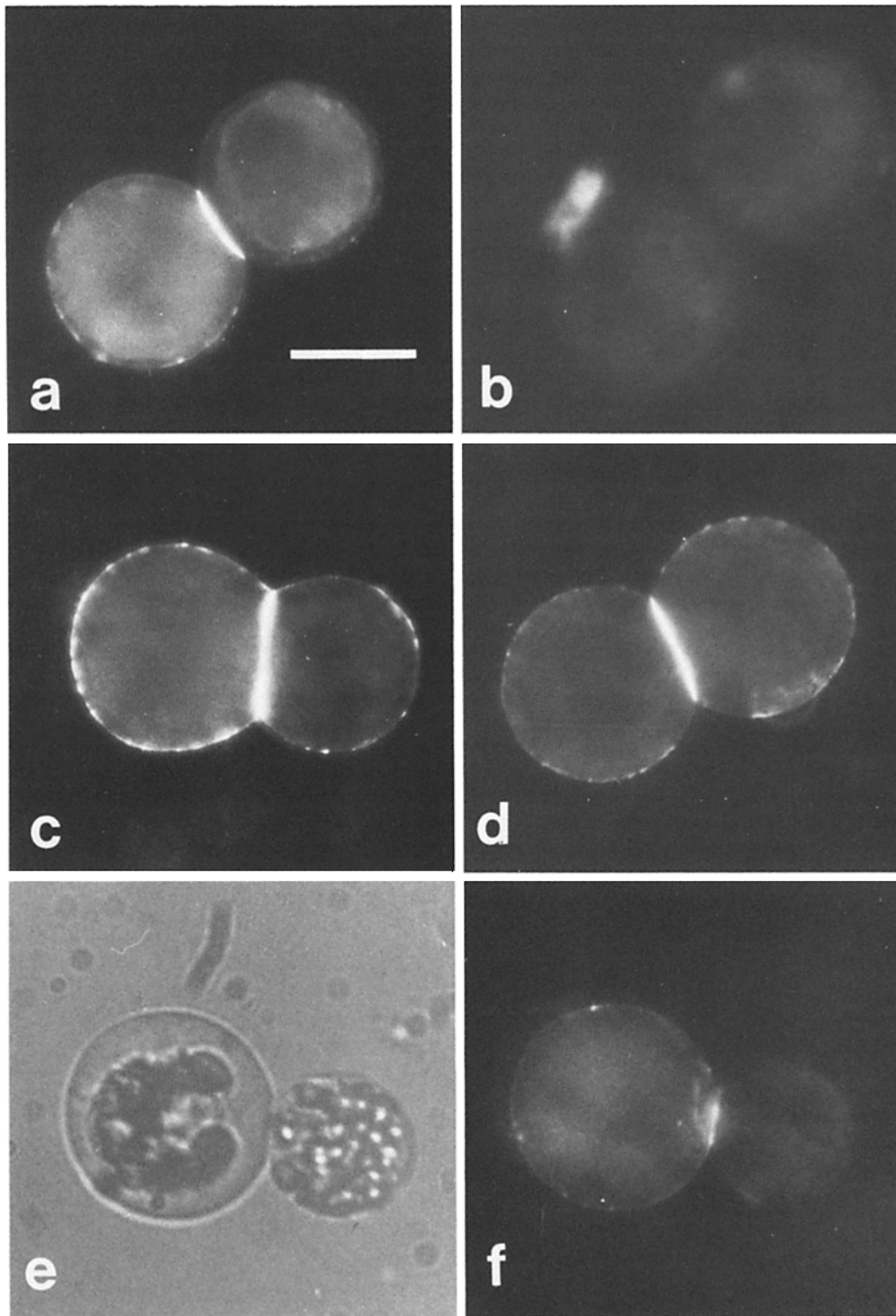
FIGURE 3 Accumulation of SBA receptor at contact site in 1-d-old *Xenopus* cultures after 30 min of contact in various experimental conditions. (*a*): Control cell pair showing high receptor accumulation at the contact site. (*b*): Cells treated identically as those for *a* except that 10 mM galactose was present throughout the experiment. The absence of F-SBA ring staining indicates that the binding of F-SBA on the surface was specific. Remaining fluorescence is due to autofluorescence of the intracellular yolk granules that are present in many of these embryonic *Xenopus* cells. (*c* and *d*): Cells treated the same as control (*a*), except that 10 mM of galactose (*c*) or glucose (*d*) was present, respectively, before and during the contact period. Differences in receptor accumulation between *c* and *d*, although hardly discernible by visual comparison of the photographs, were shown to be significant by direct microfluorimetric measurement over a large number of cells (Table II). (*e* and *f*): Bright field and fluorescence micrographs of a muscle cell in contact with a dermatomal cell, showing slight receptor accumulation. In this particular group of cultures, patching of lectin-receptor complexes was more pronounced than that of Fig. 1. Variability of patchiness, however, did not affect the measurement of the accumulation index. Bar, 20 µm. × 1,000.
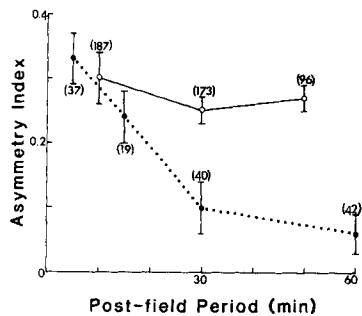
FIGURE 4   Lateral diffusion of ligand-free SBA receptors (●····●) and the relative immobility of SBA-receptor complexes (O——O) in 1-d-old *Xenopus* muscle culture. Accumulation of SBA receptors on isolated spherical muscle cells was first produced by exposure of a number of cultures to a steady extracellular electric field of 10 V/cm for 10 min. The cells in various cultures were then either labeled with F-SBA immediately after the termination of the field (O) or allowed to relax in the absence of the field for various periods of time before the labeling was carried out (●). The extent of asymmetry in SBA receptor distribution was expressed in terms of asymmetry index, which was defined as $(I_c - I_a)/(I_c + I_a)$, where I_c and I_a are corrected fluorescence intensities measured microfluorimetrically at the cathode- and anode-facing poles of the cells, respectively (see reference 17). Each data point represents the average asymmetry index measured on large numbers of cells in different parallel cultures. The error bar represents 95% confidence limits and the number associated with each bar indicates the number of cells measured for each point.

Fig. 5 *a* and *b*). As shown in Fig. 4, if the labeling with F-SBA was carried out immediately after the removal of the field, the asymmetry indices remained at high values for a prolonged period, indicating that SBA-receptor complexes remain relatively immobile on the cell surface. However, if the labeling was delayed for different periods after removing the field, the asymmetry indices decayed rapidly. Within 1 h of postfield relaxation, no significant asymmetry of SBA receptor distribution was found, indicating that ligand-free SBA receptor may undergo rapid lateral diffusion in the membrane. The rate of lateral diffusion of ligand-free SBA receptors found in the present study is similar to that reported previously for *Xenopus* muscle cells in 2-d-old cultures (3). Detailed mathematical analysis in the latter report has shown that the rate of lateral diffusion of SBA receptors is rapid enough to account for the rapid entrapment of these receptors at the contact site between the cells if the site of the contact serves as an ideal "sink" for the diffusing receptors.

Distinction Between SBA and Con A Receptors

Lectins recognize their specific sugars on cell surface molecules, and each surface glycoprotein may bind to different lectins. The fact that SBA receptor density was increased at the contact site, whereas Con A receptors appeared to have migrated away from it suggests that many SBA receptors are molecules physically separate from Con A receptors. To verify this conclusion by an independent method, we performed an experiment using the method of *in situ* electrophoretic segregation (15). This method exploits the facts that (*a*) both SBA and Con A receptors can be induced to accumulate on the surface of isolated myotomal muscle cell by an extracellularly applied electric field, and (*b*) binding of Con A to its receptors results in their immobilization (18). Cells were first exposed to an electric field of 10 V/cm for 10 min, followed immediately

by a 15-min labeling of R-Con A (25 μg/ml) at 0°C. The cells were then exposed to a second field of the same strength, but of reversed polarity for 30 min, followed by a second labeling with F-SBA. The first labeling with R-Con A immobilized all the existing Con A receptors on the surface, because reverse field produced no alteration of the asymmetric pattern of R-Con A staining, and incorporation of new Con A receptors into the membrane is negligible within the duration of this experiment (Poo, M-m., unpublished observation). The reverse field, however, resulted in an accumulation of SBA receptors on the new cathodal pole of the cell. A representative cell from this experiment is shown in Fig. 5 *c* and *d*. This result indicates that a substantial amount of the SBA receptors is physically separate from the Con A receptors, and that cell-cell contact could thus induce different patterns of redistribution for the two receptor species on the cell surface.

DISCUSSION

Contact-Induced Redistribution of Surface Receptors

In the present report, we have demonstrated that substantial redistribution of cell surface lectin receptors occurred within 30 min after contact was made between embryonic *Xenopus* muscle cells in 1-d-old cultures. SBA receptors accumulated toward the contact area, whereas Con A receptors moved away from it. A much less extensive redistribution of several other lectin receptors also occurred. The accumulation of SBA receptors at the contact area was greatly reduced by precontact binding of SBA or by lowering the temperature of the incubating medium. However, it was unaffected by treatment with metabolic inhibitors, suggesting that the redistribution was a passive process of lateral diffusion of the receptors in the plane of the plasma membrane. The accumulation of SBA receptors could have resulted from the entrapment of rapidly diffusing receptors by their binding to specific ligands on the opposing cell surface, whereas exclusion of Con A receptors from the contact area may be due to extensive accumulation of SBA and other surface receptors within the limited space of the contact area. Consistent with this notion were the findings that ligand-free SBA receptors are capable of rapid lateral diffusion in this muscle cell membrane; blocking the lateral diffusion of the SBA receptors by precontact binding with F-SBA prevents the redistribution of the receptors; and a substantial fraction of the SBA receptors is a physically separate component from the Con A receptors. Moreover, the rate of lateral diffusion of SBA receptors is fast enough to account for their rapid accumulation at the cell-cell contact site by a simple "diffusion-trap" mechanism, as shown previously for 2-d-old culture cells (3).

Other evidence suggests, however, that the accumulation of SBA receptors may also involve cellular processes other than the simple "diffusion-trap" mechanism. Pretreatment of the cells with cytochalasin B, a microfilament disrupting agent, greatly reduced the extent of accumulation induced by 30-min contact. It is possible that the integrity of submembranous microfilaments helps to maintain a high concentration of trapped SBA receptors at the contact site. If the latter process indeed occurs, its metabolic energy requirement must be extremely limited, because pretreatment with metabolic inhibitors for a prolonged period was without effect on the extensive accumulation of SBA receptors. The effect of cytochalasin B could also result from the removal of surface components
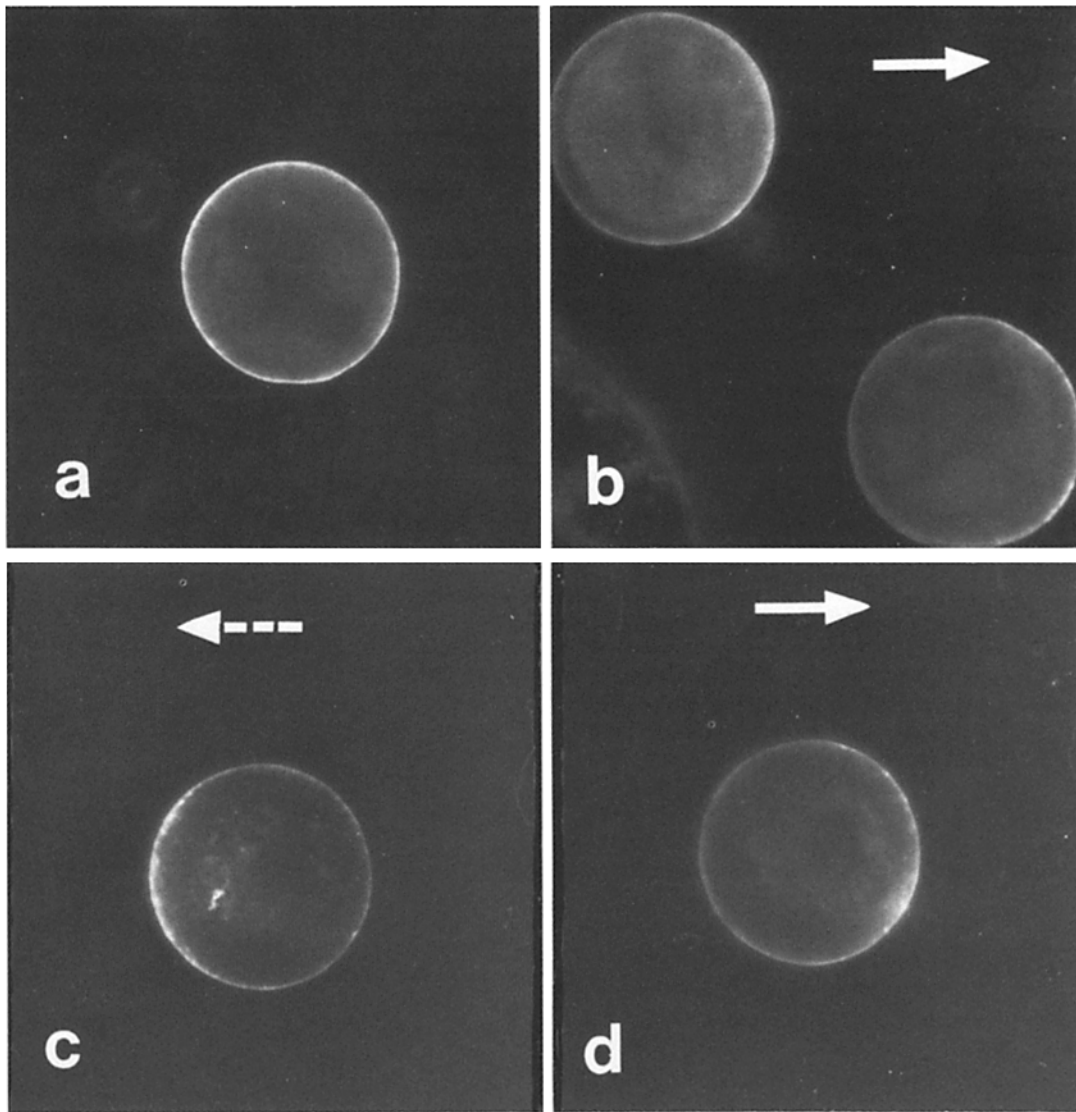
FIGURE 5 Lateral mobility and electrophoretic segregation of SBA and Con A receptors. (a): Uniform distribution of SBA receptors on a typical muscle cell in 1-d-old culture not treated with electric field. (b): An external electric field (10 V/cm) was applied to a culture for 10 min. Immediately after termination of the field, the cells were labeled with F-SBA, showing accumulation of SBA receptors at the cathode-facing pole of the cells. (c and d): A culture was first subjected to an electric field (10 V/cm, 10-min duration), followed immediately by R-Con A staining. The cells were then exposed to a second field of the same strength, but of reversed polarity (for additional 30 min), followed by a second labeling with F-SBA. The first staining immobilized the Con A receptors against the action of the second field, which moved the SBA receptors toward the opposite pole of the cell. c and d are fluorescence micrographs taken in the same focal plane of the same cell at 590 nm and 520 nm, respectively, for rhodamine and fluorescein fluorescence. Arrows indicate the direction of the externally applied electric field. × 1,000.

involved in the trapping of SBA receptors. At the concentration (10 μg/ml) of cytochalasin B used in the present study, a substantial amount of a major surface protein, LETS, was found to be released from the surface of fibroblasts within a few hours (1). Both treatments with metabolic inhibitors and colchicine, a microtubule disrupting agent, appeared to slightly increase the accumulation of SBA receptors. An energy-dependent microtubule network could normally impose a general restriction on the lateral mobility of cell surface receptors. Therefore, depletion of energy or disruption of the microtubules allowed a more effective movement and subsequent accumulation of the SBA receptors.

Finally, despite the consistency of our data with the "diffusion-trap" hypothesis, we have not ruled out the possibility that the contact-induced accumulation of SBA receptors

results from selective local insertion of new SBA receptors at the contact site and simultaneous removal of preexisting SBA receptors at the noncontact area. If this selective turnover process indeed occurs, it must operate with a rather limited metabolic energy supply. Under the condition of metabolic inhibition used in the present study, where the accumulation of SBA receptors still occurred, the cells have been shown to lose their contraction response to bath-applied acetylcholine within 1 h (18), suggesting depletion of energy supply by the treatment.

Specificity of Contact-induced Receptor Accumulation

Our evidence that contact-induced accumulation of SBA

receptors involves specific molecular interaction came from the study using SBA specific sugars, namely D-galactose and N-acetyl-D-galactosamine. When these sugars were present in the incubating medium before and during the contact period, the accumulation of SBA receptors was significantly reduced. Comparable treatment with D-glucose, a sugar specific for Con A, was not as effective in reducing the SBA receptor accumulation. This suggests that galactose and N-acetyl-D-galactosamine residues may be involved in the molecular interaction between these contacting muscle cell surfaces. However, the carbohydrate moiety responsible for the interaction that led to the accumulation of SBA receptors is unlikely to be simply galactose or N-acetyl-D-galactosamine residues. Otherwise, the cell-cell contact should have prevented the binding of fluorescent SBA to these sugars at the contact site and we should not be able to observe any receptor accumulation. Furthermore, substantial accumulation was still observed even when both SBA specific sugars were present in the incubating medium. Such treatment should have essentially abolished the simple lectin-sugar binding between endogenous SBA-like surface molecules and SBA specific sugar residues on the other cell surface (see also Fig. 3 b). The complementary molecules for the SBA receptors thus may recognize carbohydrate moiety that is in close proximity to or inclusive of the D-galactose and N-acetyl-D-galactosamine residues. Such a complementary molecule could be considered as an endogenous surface lectin with sugar specificity close but not identical to that of SBA. Interestingly, endogenous lectinlike substances have been found in cellular slime molds and a variety of animal tissues, including chick myoblasts. Their possible role in mediating cellular adhesion has been suggested (for a review, see reference 2).

The problem of specificity was forced to the forefront when we considered the fact that many other lectin receptors (e.g., those for DBA, PNA, and WGA) also accumulated at the contact site, although to a much smaller extent. Because it is likely that each group of these receptors consists of many molecules physically distinct from the SBA receptors, two possibilities exist: first, many structurally distinct receptors accumulated at the contact site, and second, a single receptor species did. If the latter is true, our observations suggest that this particular molecule must contain many different sugar residues, the relative amount of which is indicated by the extent of accumulation for various lectin receptors.

Possible Functions of Contact-induced Redistribution

Selective intercellular adhesion between homotypic cell types is a well-known characteristic of embryonic cells. It is likely that such selective adhesion plays an important role in the morphogenesis of embryonic tissue. The rapid accumulation of surface receptors at the site of cell-cell contact by the binding of these receptors with specific ligands on the opposing surface could serve as a mechanism for enhancing selective adhesion of the two surfaces. The redistribution of surface components following cell-cell contact could also provide a mechanism for inducing topographic differentiation of the plasma membrane during development (6).

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