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POLARIZED MEMBRANE DOMAINS OF FIBROBLASTS

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Abstract

Data on the heterogeneous distribution of various membrane domains on the surface of fibroblasts are reviewed. Polarized localization of negative charges is demonstrated and new results on the development and maintenance of polarity in primary human fibroblasts are presented. Cell membrane heterogeneity in fibroblasts and in other cells is compared. Our results indicate that the regional localization of membrane domains of fibroblasts, and their dependence on cell movement and cell contacts is in several aspects similar to that described for epithelial cells.

Key words: fibroblasts, membrane heterogeneity, membrane polarity, membrane domains, transmission electron microscopy, cytochemistry.

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Introduction

It is known that considerable diversity exists in the shape of fibroblasts as well as in the arrangement of cytoplasmic and surface constituents. In the present paper we review recent data on this phenomenon with special emphasis on observations concerning heterogeneous distribution of various membrane domains on fibroblast surfaces.

The shape of moving fibroblasts

Moving fibroblasts display a dorsoventral polarity, which is independent of the culture conditions (50,81,87,115). The surface consists of apical, lateral and basal membrane regions. Some of the characteristic membrane regions such as cell contacts appear basolaterally; others, such as villi or cell surface blebs are found apically (15,39,40,41,56,69,113). Along their longitudinal axes, the cells can be divided into a leading edge or lamellipodium and an elongated trailing edge (1,49,101).

Regional differences in the distribution of cytoplasmic organelles

Electron microscopic studies revealed that some of the cytoplasmic organelles of the moving fibroblasts such as mitochondria, the Golgi complex, microtubules, the microtubule organizing center have specific localizations. For example, mitochondria are accumulated closely around the nucleus, while the Golgi complex and the microtubule organizing center are placed between the nucleus and the ruffled leading edge (19,65,66,115).

Similar to other cells, the fibroblasts also contain a membrane-associated cortex of contractile elements (9) which are concentrated mainly on regions of microvilli and cell contacts, whereas they are absent in other regions (blebs) (39,74,113). These filaments are also present in the leading and trailing edges

of moving cells (103).

Regional differences in the lateral mobility of membrane components

Membrane components of morphologically different cell surface regions may differ from each other in the lateral mobilities of the major histocompatibility antigen (MHC) and that of a glycoprotein of 80 000D (GP 80) (55,58,121). The mobility of leading and trailing edges is different. The ratio of the diffusion constants is 1.9 for MHC and approximately 3 for GP 80 (58,121).

The mobility of membrane components in the blebs is higher than in other membrane areas (74). In contrast, lateral mobilities of certain lipids and proteins at cell contacts are decreased as compared to other membrane areas and as a consequence, the amount of the immobile protein fraction is significantly increased (7,29, 40,78,121).

Regional distribution of membrane domains on the fibroblast surface

The distribution of lipid, protein carbohydrate domains as well as that of the receptors and ligand binding sites is not homogeneous (25, 39, 44, 61,92). It is frequently observed that certain domains are localized at preferred regions of the cell surface. Such functional and structural inhomogeneities of the cell membrane are well known phenomena in epithelial cells (52,72,76,90,93,98). Recent observations, however, suggest that membrane polarization also exists in fibroblasts. Thus, the surface glycoprotein GP 80 is localized predominatly on the trailing edge (55,58), whereas GP 90 and GP 100 are present in the coated pits and in the cell contacts, respectively (77). The urokinase-type plasminogen activator is known to reside in the cell contacts (51,84). According to our recent experimental data, also adenylate- and guanylate-cyclase display a polarized distribution in primary human fibroblasts (108). Adenylate cyclase present on the baso-lateral membranes of cells (108) just like in human trophoblasts, urinary bladder epithelial cells and enterocytes (24,70, 73). On the other hand guanylate cyclase is localized in the apical region of the fibroblasts (Fig.1) similar to its localization in human trophoblasts (73). There are also some data about the inhomogeneous distributions of cell surface receptors for GM 3 hematoside (94), LDL (80,95), macroglobulin (47), transferrin (32) and H 2 antigen (80) in fibroblasts. Polarized distribution of lectin binding sites in primary human fibroblasts has also been observed. According to our observations Concanavalin A (Con A) binding

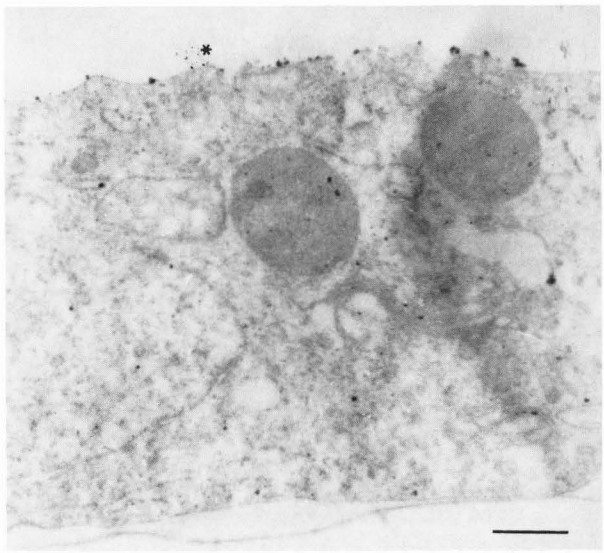


Figure 1. Distribution of guanylate cyclase on a fibroblast surface. The section was not stained. The enzyme is localized on an apical surface of the cell (*) and is completely absent on the basal area. Bar = 0.5 /um.

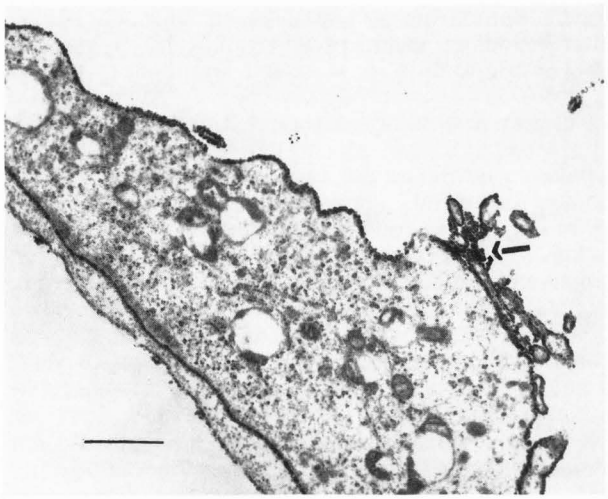
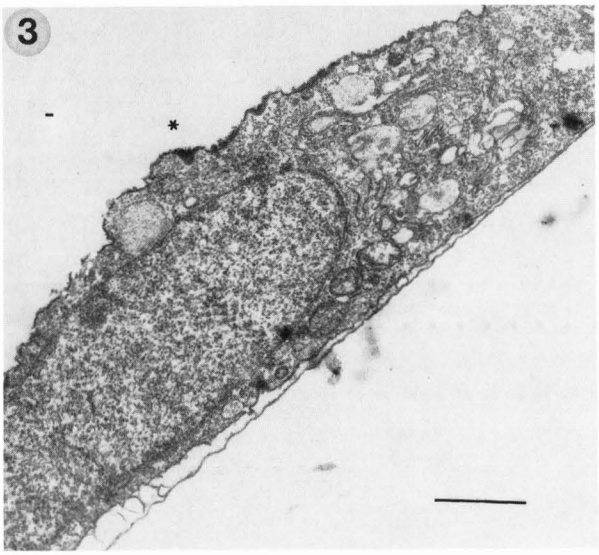
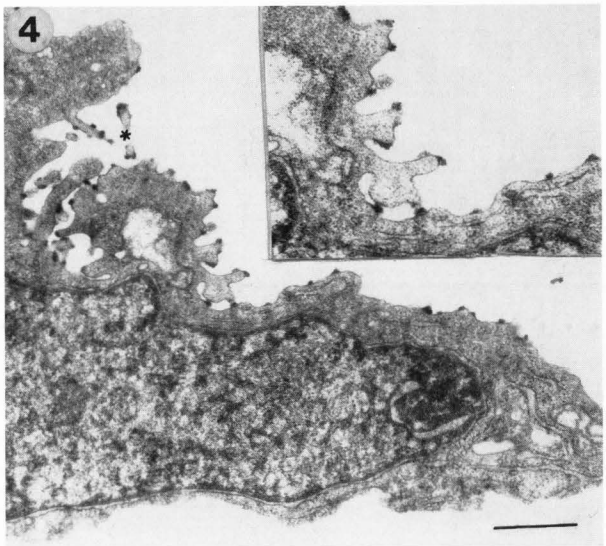


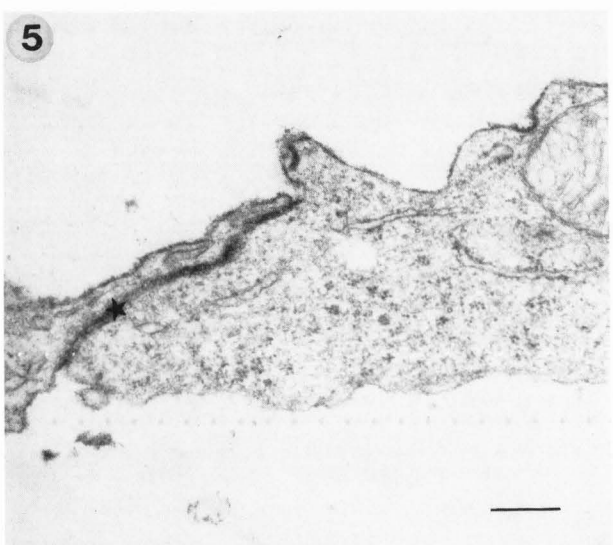
Figure 2. Concanavalin-A binding on primary human fibroblasts. The reaction products can be seen on the whole surface. The amount of Concanavalin-A binding sites is increased at the sites of cell contacts (⟶). Bar = 0.5 /um.

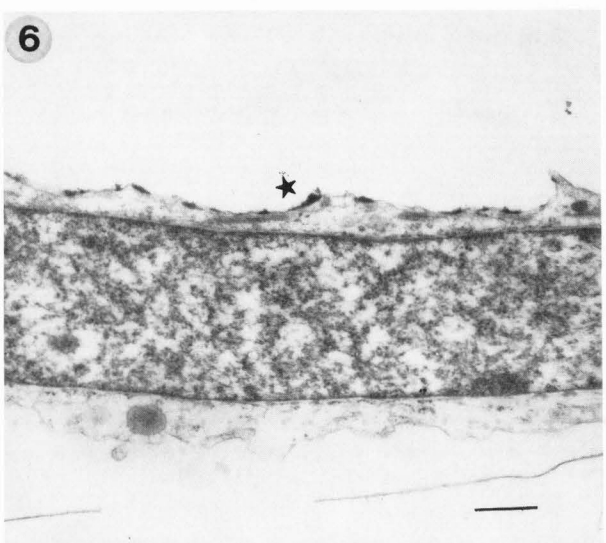
sites occur on the whole surface of fibroblasts (107) (Fig. 2). Wheat germ agglutinin (WGA) binding sites are localized on the apical membrane regions (107) (Fig.3).

Several reports show that the distribution of binding sites for extracellular components, i.e. hyaluronic acid









(116), heparin sulphate (117) and bound fibronectin (15,29,31,35,75) also is inhomogeneous. In addition, specialized membrane regions such as cell contacts have specific structural and/or regulatory proteins (2,13,14,38,41,79,100).

Data are available on the preferred apical localization of negative charges on the surface of fibroblasts (8,42,46, 105,106) (Figs. 4-5-6). Accumulation of negatively charged groups is also characteristic for the cell-to-cell contact regions (46,106) (Fig. 5).

Microvilli and other cytoplasmic projections display a more pronounced negative charge than surrounding surface areas of BHK cells (46), primary human fibroblasts (106) or L 49 cells (Fig. 4).

The polarized virus budding, which phenomenon is a usual model to investigate epithelial membrane polarity (91,93,99), also occurs in L-mouse fibroblasts (12).

Figure 3. Binding of WGA on the apical region of primary human fibroblast (*). Bar = 0.5 μm.

Figure 4. The distribution of negative charges on L 49 fibroblasts as visualized by cationized ferritin. Negative charged sites occur on the apical surface and its more pronounced microvilli and other cytoplasmic projections (*). Bar = 1 μm.

Figure 5. Cationized ferritin binding on primary human fibroblast. Heavy binding of ferritin is seen at the cell contacts (*). Bar = 1 μm.

Figure 6. Cationized ferritin binding on primary human fibroblast cultivated on glass substrate before trypsin treatment. Ferritin binding is seen exclusively on apical surface (*). Bar = 0.5 μm.

Data demonstrating the inhomogeneous distribution of membrane components in different types of fibroblasts are presented in Table 1.

Correlation between the functional state of cells and the heterogeneity of plasma membrane

Membrane inhomogeneities, which are characteristic for moving cells may disappear after cell locomotion ceases. This was shown for GM 3 hematoside, transferrin, GP 80 and hyaluronic acid binding sites (32,57,94,116).

The polarized insertion of G protein of VSV virus occurs only in moving cells (67,101).

In migrating fibroblasts, the cell substrate attachment (CSAT) antigen and the fibronectin receptor are diffusely distributed over the whole cell surface, but in settled cells they are located mainly in the focal contact areas (16,22, 28,29).

Possible mechanisms for maintaining the inhomogeneous distribution of membrane domains

There are data according to which the membrane polarity in fibroblasts is maintained by at least two mechanisms just like epithelial cells (59). Polarized insertion of membrane compartments and/or their immobilization in the plane of the membrane may maintain the inhomogeneous distribution of membrane domains.

The first mechanism is well documented for epithelial cells (11,43,61,120). A known example of this phenomenon is the polarized insertion of the G protein of the vesicular somatitis virus in the leading edge of normal renal fibroblasts (5).

Polarized membrane insertion is basically dependent on active locomotion of the cells (5,67,101).

According to some experimental data, the immobilization of membrane domains is brought about by anchorage through microfilamental and microtubular systems. Even if the domains are not immobilized completely, their movements are limited by the diffusion barriers between different membrane regions. Thus, it is known that the fibronectin receptor involved in a transmembrane linkage is connected to extracellular fibronectin fibers and intracellular actin filaments (17,29,99). The clustered localization of LDL receptors in coated pits can also be explained by the immobilization of domains through the cytoskeletal system (3,95). A possible linkage between the extracellular plasminogen activator and the contractile elements in the fibroblast membrane was recently suggested (51). Special cell surface regions i.e. contacts are built up by intracellular membrane proteins (13-15,68,86, 122) and are stabilized by bundles of microfilaments (9,39,60,63,92,94,103).

The cell contacts of epithelial cells are known to create diffusion barriers within the membranes and in this way maintain membrane heterogeneities (25,52,59, 93,114). Therefore, it appeared reasonable to study the effects of cell contacts on membrane polarity also in fibroblasts. Since the localization of the negatively charged sites is known both for fibroblast and epithelial cells (37,40,105, 109) and can be easily observed by the cationized ferritin binding method, the changes of this distribution were followed in our studies on primary human embryonic fibroblast cultures.

Materials and Methods

Cell culture

Experiments were performed on semiconfluent primary embryonic human fibroblast and L 49 mouse fibroblast cultures. L 49 cells were routinely grown in monolayer TC 199 Parker medium supplemented with 10% calf serum. Details of fibroblast isolation and culture were described in a previous paper (107).

Detached and suspended fibroblasts

Cells were suspended by mild trypsin treatment (0.5% trypsin, 1 min). Cells detached from the culture dish and washed under sterile conditions at room temperature were centrifuged at low speed, and then resuspended in complete culture medium by continuous shaking. 50-70% of cells remained viable after 24 h as shown by Trypan Blue staining. Samples were taken from the cultures 15 min, 1,2,3,6 and 24 h after suspending. Cells in the samples were fixed while still suspension and sedimented on coverslips or put into glass Petri dishes where the attached cells were fixed in situ after 15,30 to 60 minutes of plating. The fixed samples were labelled with cationized ferritin and prepared for electron microscopy in the same way as the monolayer cultures.

Cytochemistry

Surface sites with negative charges were detected by cationized ferritin (CF) according to the method of Danon et al (23). For the detection of Concanavalin-A bound to glucose and mannose side-chains, the Concanavalin-A-peroxidase reaction was used (6). The sialic acid and N-acetyl-glucosamin side chains bound to wheat germ agglutinin (WGA) were visualized by glucose oxidase (34). The specificity of Con-A and WGA binding was controlled with alpha-D-mannose and N-acetyl-glucosamine.

For cytochemical localization of guanylate cyclase activity the guanydylimidophosphate method (GMP-PNP) (119) was used.

The cells were previously fixed with 0.05% glutaraldehyde in all experiments. All reagents used were from Sigma Co.

Table 1.

Regional membrane domains on the surface of fibroblasts

Membrane domain	Localization	Cell type (functional state)	Reference
80 000 D major cell surface glycoprotein (GP 80)	dominantly on the trailing edge, mainly on the leading edge	C3H/10T[1] cells. log. phase	57
90 000 D major cell surface glycoprotein	in the coated pits	NIH/3T3 cells	77
100 000 D major cell surface glycoprotein	cell contacts	NIH/3T3 cells	77
urokinase type plasminogen activator	cell contacts	HT 1080 fibroblasts	84,85
adenylate cyclase	basolateral	skin and primary human embryo fibroblasts	108
guanylate cyclase	apical	primary human embryo fibroblasts	present paper
GM 3 hematosid binding sites	dominantly on the leading edge	NillCl secondary hamster fibroblasts, log phase	94
low density lipoprotein (LDL) receptors	diffusely on the leading edge, in coated pits on other surface areas	human skin fibroblasts	95
L-2 macroglobulin receptors	in the coated pits	3T3, rat fibroblasts	47,95
H-2 antigen receptor	label only on the cell body, absent from the microvilli	mouse L cells	80
Trypanosoma receptor	on the cell edge	3T3/A31 fibroblasts	96
Con-A binding sites	less on the microvilli, pronounced at the cell contacts	3T3 fibroblasts, primary human fibroblasts	20 present paper
wheat germ agglutinin binding sites	apical	primary human fibroblasts	107
surface negative charges	apical, cell contacts, microvilli	primary human fibroblasts, mouse embryo fibroblasts, BHK cells, L49 fibroblasts,	105,106 42,46 present paper
hyaluronic acid binding sites	dominantly on leading edges	chicken heart fibroblasts, log phase	116
heparin sulphate binding sites	clustered, cell edges, cell contacts (extracellular protein in focal contacts)	rat embryo fibroblasts	122
fibronectin binding sites	basal[+], clustered, at the cell contacts[++], interconnecting networks of randomly oriented fibrils, cell contacts (structural protein in focal contacts)	BHK cells, young human fibroblasts, S142, fibroblasts, Detroit 551 fibroblasts, old human fibroblasts, confluent human fibroblasts	45,31 82,83,118 15,75 31,35 29
Other structural and regulatory proteins in focal contacts	cell contacts	chicken fibroblasts	13,14,104

[+] this polarity was observed only in cells attached to substrate containing fibronectin (45)

[++] distribution was not changed by culture density (31)

Transmission electron microscopy

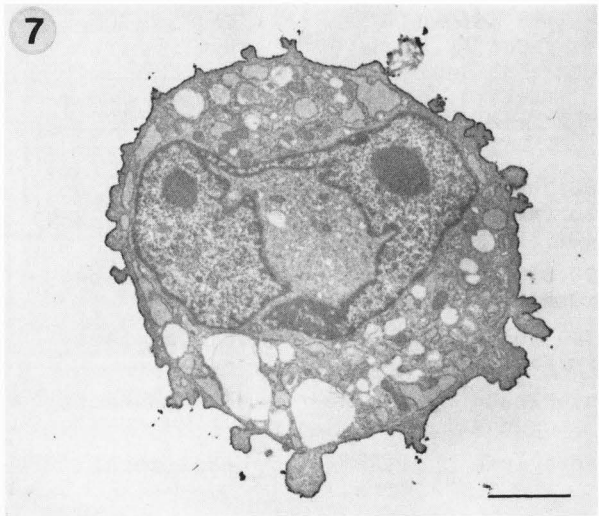
The cells were fixed on coverslips in 2.5 per cent glutaraldehyde followed by 1 per cent OsO_4 in 0.1 M phosphate buffer, pH 7.3 at 4°C for 1 h each. The material was dehydrated with acetone and embedded in Durcupan AC (Fluca).

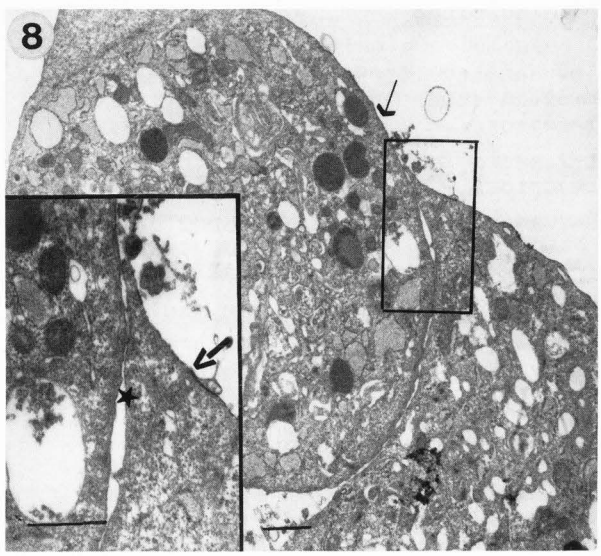
Ultrathin sections were cut with diamond or glass knives on an LKB ultramicrotome in an orientation perpendicular to and parallel with the plane of the cell culture. The sections were stained either with uranyl acetate and lead citrate or left unstained, and examined with a JEM 6C electron microscope.

Cell contacts and polarity of cationized ferritin binding in fibroblasts

The primary human fibroblasts dissociated from the monolayer by trypsin and kept over 24 h in suspension culture, were usually rounded up and covered by blebs (Fig. 7). In contrast to the cells in the control monolayer, where the CF bound apically to the plasma membrane of the fibroblasts (Fig. 6), negatively charged sites appeared on the whole surface of suspended cells (Fig. 7). This distribution of CF binding sites was observed on the cells 1-24 hours after isolation. However, cell aggregates were also found frequently in the suspension and the distribution of CF in membranes of aggregated cells was different from that in isolated cells. In this case, cationized ferritin binding was observed only on the free cell surfaces but not on adjacent cell membranes (Fig. 8). Cells taken from the suspension can easily attach again to the substrate and at the same time the polarization of their negative surface charges reappears.

One hour after re-attachment the cells became flattened and their morphological appearance as well as their binding characteristics for cationized ferritin became similar to those of the cells before treatment (Fig. 9). Similar experimental data were reported for dissociated





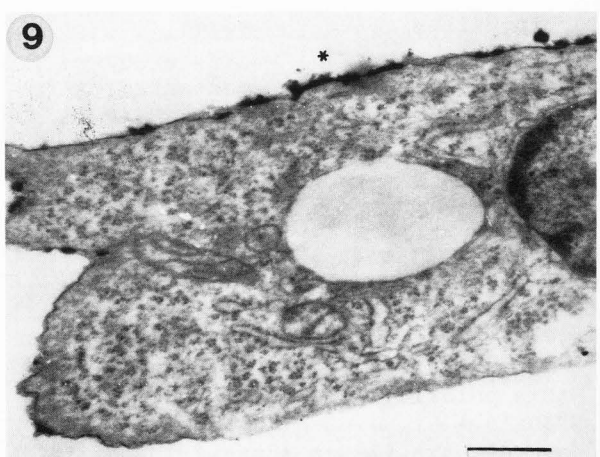


Figure 7. Single fibroblast after 3 h of trypsin treatment. Ferritin is localized all over the surface of the rounded cell. Bar = 1 μm.

Figure 8. Cationized ferritin binding on a group of cells 3 h after the trypsin treatment. The free cell surface was labelled by ferritin (➡), no labelling occurred on the cell contact region (✱). Bars = 0.5, insert 0.1 μm.

Figure 9. Cationized ferritin binding after shaking of the cells for 1 h. The ferritin particles are present on the apical and lateral surfaces (✱), but are absent on the basal one. Bar = 0.5 μm.

Table 2.

The occurrence of membrane domains in regional distribution on other cell types

Membrane domains	Epithelial cells	Other cell types
urokinase type plasminogen activator	in the clusters: (A-430 human epidermoid carcinoma (33)	
adenylate cyclase	inhomogeneous: mammary adenocarcinoma (53), basolaterally (frog urinary) bladder (24), human trophoblasts (73)	rat cardiac muscle: gap junctions (36)
guanylate cyclase	inhomogeneous: mammary adenocarcinoma(53), apical: human trophoblasts (73)	rat cardiac muscle: gap junctions (36)
peripheral membrane proteins associated to junctional elements	MDCK cell junctions(100), rat kidney epithelium (27, 110)	rat ascites hepatoma (48)
fibronectin binding sites	basally, at the cell substrate contacts (27), embryonal corneal epithelium (112), M21 melanoma cells (18)	
proteoglycan binding sites	basolaterally: in confluent, NMuMG, mouse breast (54), epithelium (88)	
heparin-sulphate proteoglycan	basally: at the cell contacts (68), on the microvilli: human colon carcinoma cells (58)	
wheat germ agglutinin binding sites	apical: porcine thyroid gland cells (4), HeLa cells (71)	polarized: chemotactic monocytes (62), neuromuscular junctions (89)
Concanavalin-A binding sites	epidermis cell junctions (97)	neuromuscular junctions (89), polarized: moving thymocytes (26), monocytes (62,102), localized head of the acrosomal region: mammalian spermatozoa (30)
negatively charged sites	apical: chicken retina pigmented epithelium (21), frog urinary bladder epithelium (37), HeLa cells (71)	polarized: lymphoid leukemic cells (64), cardiac muscle gap junctions (36), on the microvilli: Ehrlich ascites cells (111)
GM3 hematosid binding sites	predominantly on the leading edge: B16 melanoma (94)	
low density lipoprotein (LDL) receptors	with diffuse distribution at the cell margins, clustered in the central region: subconfluent calf aorta endothelium, E8 cells (95), leading edge: giant HeLa cells (10)	on the intermediate surface regions, macrophage (90)
actin depolymerizing factor		in the microspikes and along the neurite shaft: cultured dissociated dorsal root ganglia cells (2)
Trypanosoma cruzi binding sites	confluent MDCK cells on the basolateral membranes, sparsely plated cell on the edges (96)	

frog urinary bladder epithelial cells (37) in which 15 min after dissociation, negatively charged sites were found on the entire surface of rounded cells. The ability for polarized virus budding in Madin-Darby Canine kidney cells was lost in suspension cultures. However, the aggregated cells have membrane inhomogeneities as indicated by polarized virus budding (91). Taken together, the observations presented here and other reports (see Table 1) strongly support the view that polarized distribution of surface components exists in fibroblasts and is sensitively regulated by the functional state of the cell. Evidence for the existence of membrane inhomogeneities in epithelial cells and cell types is presented in Table 2.

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Discussion with Reviewers

Reviewer III: What precautions did the authors take to control for the non-specific binding or trapping of the ferritin markers in regions of close cell-cell apposition?

Authors: The cationized ferritin is often used as a marker for electron microscopic visualization of negatively charged sites on cell surface. We did not find any refference in the literature according to non-specific binding of this marker. At the same time we have also checked in our experiments the characteristic localization and specificity. In addition, we have examined the distribution of ruthenium red, as another marker of negatively charged sites. In both cases we experienced similar staining patterns as described in the text.

Reviewer III: What changes in ferritin distribution occur if cells are prelabeled in suspension and then plated out and allowed to spread? Are anisotropic distributions seen before they come into contact?

Authors: In the suggested experimental situation the cells would take up the marker (42), thus the examination of the change in distribution of ferritin particles by this way seems to be impracticable.