CELL ADHESION ON MODEL SUBSTRATA: THRESHOLD EFFECTS AND RECEPTOR MODULATION

J. D. APLIN AND R. C. HUGHES*
National Institute for Medical Research, Mill Hill, London NW7 1AA, U.K.

SUMMARY
Trypsinized BHK cells become attached to glass that has been derivatized with a variety of lectins with well-defined specificity for cell-surface carbohydrates. Provided a threshold concentration of glass-immobilized protein is present the cells undergo a transformation to a well-spread morphology. The matrix density of lectins (ricin and concanavalin A) required to trigger this morphological transformation is higher by 10 to 40-fold than the value determined earlier (Hughes, Pena, Clark & Dourmashkin, 1979) for fibronectin.

Cells resistant to the toxic lectin, ricin, and expressing 10% or less of ricin-binding carbohydrate groups at their cell surfaces require correspondingly greater matrix densities of ricin to promote active cell spreading. All cell lines spread equally well on concanavalin A-based matrices consistent with their similar binding properties.

The quantitative interaction of complementary molecules on the cell surface and matrix, promoting cell adhesion, is demonstrated by these results and a model is proposed for the events leading to a well-spread cell morphology on a protein-coated substratum.

INTRODUCTION
Cellular adhesion to other cells or to inert substrata such as connective tissue elements is a fundamental property of cells in multicellular organisms and plays an important role in initiation of growth of anchorage-dependent cells, differentiation and cell motility. Adhesive interactions may occur through the binding of complementary molecules expressed on the surface of apposed cells (Tyler, 1946; Weiss, 1947) or between the underside of substratum-attached cells and the molecules of the substratum (Grinnell, 1978). In several systems cell-surface carbohydrates have been implicated in these adhesive interactions although unequivocal proof is still lacking (Hughes, Pena & Vischer, 1980). Still less is known about the quantitative basis of cellular adhesion; namely, how many interactions of the type proposed by Tyler & Weiss are required to stabilize interactions.

Recently, there has been considerable interest in the potential of artificial or synthetic surfaces for the study of cell adhesion and in determining the membrane properties required for normal adhesive behaviour (Chipowsky, Lee & Roseman, 1973; Schnaar et al. 1978; Weigel et al. 1979; Sugimoto & Hagiwara, 1979; Fisher & Solursh, 1979; Vlodovsky, Lui & Gospodarowicz, 1980; Carlsson et al. 1979; Iwig, Lasch & Glasser, 1980).

* Author to whom correspondence should be sent.
Fibronectins, a class of glycoproteins of approximate molecular weight 440,000 and present in mammalian body fluids, in cell surfaces and in association with connective tissues, promote cell adhesion to otherwise inert surfaces (Pena & Hughes, 1978; Grinnell & Hayes, 1978a, b; Grinnell, 1978; Hughes et al. 1980). After attachment to substrate-attached fibronectin the cells undergo a change in morphology from an initially rounded appearance with convoluted surfaces and become well-spread, the cytoplasm thin and the surface relatively smooth when examined by electron microscopy. We (Hughes et al. 1979a, 1980) and others (Grinnell & Hayes, 1978a, b) have shown that these morphological changes induced by fibronectin, present as a surface coating in assays in vitro, can be mimicked by other proteins known to recognize the cell surface, such as concanavalin A and antibodies, as well as less specific surface-binding moieties such as polycationic ferritin.

In the present studies we extend these observations to other model substrata. We demonstrate that, as for fibronectin (Hughes et al. 1979a), a threshold density of lectin is required to promote either attachment or flattening of cells. Furthermore, a relationship exists between this matrix property and receptor numbers on the cell surface, as implied also by the prior observation that alterations in surface glycoproteins in lectin-resistant baby hamster kidney (BHK) cells affect adhesive behaviour on fibronectin (Pena & Hughes, 1978), thus providing experimental evidence supporting the principle of complementarity in the adhesion of BHK cells.

MATERIALS AND METHODS

Materials

Lectins were obtained from Miles Laboratories, Stoke Poges, U.K. Rat α1-macroglobulin was a generous gift from Dr M. J. Geisow. Laminin, isolated from mouse Reichert membranes was donated by A. Cooper and Dr B. Hogan. Bovine serum albumin and galactose oxidase were from Sigma Corporation, Poole, Dorset, U.K. Newborn calf serum was supplied by Flow Laboratories, Irvine, Ayrshire, U.K. Fibronectin was purified from newborn calf serum as described (Pena, Mills, Hughes & Aplin, 1980). Neuraminidase (Vibrio cholerae) was from Boehringer, Mannheim, Germany. Antibodies directed against the BHK cell surface were raised in rabbits as described (Hughes et al. 1979a). Before use the antiserum was passed through a gelatin–Sepharose affinity column to remove fibronectin and the immunoglobulin fraction was prepared by precipitation with sodium sulphate.

Methods

Baby hamster kidney cells and the ricin-resistant (Ric5) cell lines 14, 15 and 21 were grown in Dulbecco's modified Eagle's medium supplemented with 10% foetal calf serum and subcultured by trypsinization as previously described (Butters, Devalia, Aplin & Hughes, 1980). Cells used for adhesion assays were trypsinized in the same way, washed once with serum-free medium and suspended (10^6–10^7 cells ml^-1) in the same medium. In some experiments, suspended cells were treated for 0.5 h at 37 °C with neuraminidase (0.02 U ml^-1), washed once with serum-free medium and used at once for adhesion assays.

Lectins were radio-iodinated using the iodogen procedure and purified by affinity chromatography on appropriate supports with elution by sugar hapten (Butters et al. 1980). After dialysis against phosphate-buffered saline, the lectins were adjusted to approx. 1.0 mg ml^-1 and 10^6–10^7 counts min^-1 ml^-1.

Protein derivatization of glass coverslips (11 mm x 22 mm) was effected by treatment with 3-aminopropyltrimethoxysilane followed by glutaraldehyde, and the protein of choice. The pro-
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Procedure will be described in detail elsewhere. To quantitate the amount of covalently attached lectin, derivatization was performed using $^{131}I$-labelled lectins and after thorough washing in phosphate-buffered saline the coverslips were counted for radioactivity in a gamma-spectrometer. After covalent linking of proteins to be tested, the glass coverslips were soaked in bovine serum albumin solution (0.1 mg ml$^{-1}$) at 20 °C for 1 h and then stored at 2 °C under phosphate-buffered saline. Albumin-coated coverslips were inactive alone in mediating cell attachment or spreading. Full details will be published elsewhere (Aplin & Hughes, unpublished data).

RESULTS

BHK cell spreading on various substrata

Table 1 shows the results of adhesion tests on trypsinized BHK cells in serum-free medium using a variety of matrix-linked proteins. In addition to serum, which contains fibronectin as well as other adhesive factors (Hoffmann, Riston, Veser & Frank, 1973; Whateley & Knox, 1980), it can be seen that several proteins known to recognize cell-surface determinants are also capable of mediating cell attachment and spreading, though quite high protein concentrations are required in some cases for derivatization, thus ensuring a high surface density of immobilized protein on the glass substratum (Aplin & Hughes, unpublished data).

The lectins, concanavalin A, ricin and wheat germ agglutinin, as well as antibodies directed against the BHK cell surface, all successfully mediate cell adhesion to derivatized coverslips. The lectins, soybean agglutinin and peanut agglutinin, which do not bind to BHK cells until the latter are treated with neuraminidase (Rosen & Hughes, 1977), also mediate attachment and spreading of neuraminidase-treated cells (Table 1).

Taking the results as a whole, it is reasonable to propose that a certain number of cell membrane–matrix interactions may be required to establish a well-spread morphology of trypsinized BHK cells, and that incomplete adhesion may arise either from deficiencies in membrane-associated receptors or due to an insufficiently high surface density of matrix-attached proteins, even when very concentrated solutions are used to perform the derivatization.

The following substances failed to mediate cell adhesion when covalently attached to glass coverslips: fucose-binding protein, $\alpha_2$-macroglobulin, galactose oxidase (using trypsinized BHK cells only), and polylysine (Table 1). The fucose-binding protein (FBP) interacts with glycoproteins carrying $\alpha$-L-fucose in the oligosaccharide core region (Pereira & Kabat, 1974) and we do not know the number of binding sites (if any) for FBP on BHK cells, but the adhesion tests predict that there are not many. The same may be true for $\alpha_2$-macroglobulin, which we have nevertheless shown to interact with BHK cells and to be taken up into the cells by accelerated (receptor-mediated) adsorptive endocytosis (Aplin & M. J. Geisow, unpublished results). Thus, the possibility exists that not all recognition events occurring at the cell-surface membrane are capable of mediating cell adhesion, even if sufficiently numerous. This possibility is under study.

Our observation (Table 1) that laminin can mediate fibroblast–glass adhesion is contrary to other published data (Terranova, Rohrbach & Martin, 1980); further investigations are required to clarify this discrepancy but 2 points may be made.
**Table 1. Proteins mediating attachment and spreading of trypsinized BHK cells**

<table>
<thead>
<tr>
<th>Substratum</th>
<th>Cells</th>
<th>Maximum protein concn used to derivatize substratum (mg ml⁻¹)</th>
<th>Inhibitory substance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glass (no protein)</td>
<td>-</td>
<td>-</td>
<td>10.0</td>
</tr>
<tr>
<td>BSA</td>
<td>-</td>
<td>-</td>
<td>10%</td>
</tr>
<tr>
<td>Calf serum</td>
<td>4+</td>
<td>4+</td>
<td>0.15</td>
</tr>
<tr>
<td>Calf serum fibronectin</td>
<td>4+</td>
<td>4+</td>
<td>2.0</td>
</tr>
<tr>
<td>Concanavalin A</td>
<td>4+</td>
<td>ND</td>
<td>1.5</td>
</tr>
<tr>
<td>Ricin (RCAII)</td>
<td>4+</td>
<td>ND</td>
<td>2.0</td>
</tr>
<tr>
<td>Soybean agglutinin</td>
<td>-</td>
<td>3+</td>
<td>0.1</td>
</tr>
<tr>
<td>Peanut agglutinin</td>
<td>(+)</td>
<td>2+</td>
<td>Galβ1,3GalNAc</td>
</tr>
<tr>
<td>Fucose-binding protein (Lotus tetragonolobus lectin)</td>
<td>-</td>
<td>-</td>
<td>Fucose</td>
</tr>
<tr>
<td>Wheat-germ agglutinin</td>
<td>3+</td>
<td>3+</td>
<td>GalNAc, (NANA)</td>
</tr>
<tr>
<td>α₂-macroglobulin</td>
<td>-</td>
<td>ND</td>
<td></td>
</tr>
<tr>
<td>Galactose oxidase</td>
<td>-</td>
<td>(+)</td>
<td>15.0</td>
</tr>
<tr>
<td>Anti-BHK immunoglobulins</td>
<td>4+</td>
<td>ND</td>
<td>6.4</td>
</tr>
<tr>
<td>Polylysine</td>
<td>-</td>
<td>-</td>
<td>Galactose</td>
</tr>
<tr>
<td>Laminin</td>
<td>4+</td>
<td>ND</td>
<td>0.1</td>
</tr>
</tbody>
</table>

Trypsinized BHK cells were suspended in medium without serum and added to glass cover slips derivatized covalently with the various proteins. Cell spreading was assayed microscopically (see Fig. 2) after 2 h. Where required, cells were treated for 0.5 h with neuraminidase (0.02 U. ml⁻¹) at 37°C. ND, not determined. GalNAc, N-acetylgalactosamine; GlcNAc, N-acetylgalactosamine.
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Laminin may not be active in promoting fibroblast adhesion in test systems involving its presence in solution as well as in the substratum, since at high concentrations it agglutinates BHK cells (our unpublished results), presumably before contact of cells with substrate-attached laminin can occur. Secondly, high concentrations of matrix-associated laminin seem to be required to promote BHK cell adhesion (Table 1), so that simple non-covalent adsorption of the protein to an inert surface, as demonstrated by others (Terranova et al. 1980), may give insufficient surface-associated protein. Certainly we agree that laminin may be expected to promote adhesion of epithelial-type cells more efficiently than fibroblasts since it is in association with the former that it is found in vivo (Rohde, Wick & Timpl, 1979).

Table 2. Surface densities of immobilized protein required to promote half-maximal cell spreading in different cell lines

<table>
<thead>
<tr>
<th>Cell line</th>
<th>Substance</th>
<th>Threshold for spreading</th>
<th>Binding sites</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wild-type BHK</td>
<td>Fibronectin</td>
<td>$5 \times 10^4$</td>
<td>30</td>
</tr>
<tr>
<td></td>
<td>Con A</td>
<td>$6 \times 10^4$</td>
<td>90</td>
</tr>
<tr>
<td></td>
<td>Ricin</td>
<td>$2 \times 10^8$</td>
<td>0.18</td>
</tr>
<tr>
<td>Ric$^{14}$</td>
<td>Fibronectin</td>
<td>$3 \times 10^6$</td>
<td>9</td>
</tr>
<tr>
<td></td>
<td>Con A</td>
<td>$6 \times 10^4$</td>
<td>18.7</td>
</tr>
<tr>
<td></td>
<td>Ricin</td>
<td>$2-3 \times 10^7$</td>
<td>&gt; 2</td>
</tr>
<tr>
<td>Ric$^{21}$</td>
<td>Fibronectin</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td></td>
<td>Con A</td>
<td>$6 \times 10^4$</td>
<td>13</td>
</tr>
<tr>
<td></td>
<td>Ricin</td>
<td>$2-3 \times 10^7$</td>
<td>&gt; 5</td>
</tr>
</tbody>
</table>

* Expressed in protein molecules (440000 dimers of fibronectin; 110000 tetramers of concanavalin (Con A); 60000 monomers of ricin) per spread cell area, assuming $4 \times 10^8$ cells cm$^{-2}$ (Hughes et al. 1979a) in each case. The concentrations of ricin ($\mu$g ml$^{-1}$) required to inhibit colony formation by 90% ($D_{10}$) and the numbers of lectin surface binding sites per cell are taken from previous data (Meager et al. 1975, 1976).

The negative result obtained with glass-attached polylysine (Table 1) is noteworthy since most cell surfaces have a net negative charge, and a non-specific electrostatic interaction presumably occurs. These interactions are apparently incapable of attaching cells stably to a matrix and promoting cell spreading in the present system.

Dependence of cell spreading on the concentration of matrix-attached proteins

We have shown (Hughes et al. 1979a) that maximal spreading of trypsinized BHK cells on a plastic surface requires a minimum surface density of about $1.8 \times 10^{10}$ fibronectin molecules per cm$^2$ of substratum. Cells become attached weakly to plastic-coated surfaces with lower amounts of fibronectin and are rounded in appearance. From the area covered by a well-spread BHK cell, grown to confluence in serum-containing medium, we calculate that about 50000 fibronectin molecules may be in contact with a single well-spread cell (Table 2).

To explore further the relationship between the recognition capacity of the substratum and its ability to promote active cell attachment and spreading, we investigated...
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in detail the adhesive behaviour of trypsinized BHK cells when plated onto surfaces containing various concentrations of the lectins, concanavalin A and ricin. As shown in Figs. 1, 2A, B and Figs. 3, 4, respectively, the triggering of attached BHK cells into a well-spread morphology by ricin or by concanavalin A requires in each case a minimum or 'threshold' concentration of matrix-attached lectin. Cells were almost completely in the well-spread form on matrices carrying approximately \(8 \times 10^{11}\) or \(2 \times 10^{11}\) molecules, respectively, of each lectin per cm\(^2\) of substratum. Using the same calculation as described above for fibronectin, these values are converted to approximately \(2 \times 10^8\) and \(6 \times 10^8\) ricin molecules and concanavalin A molecules, respectively, for the matrix density of lectins underlying each spread cell. It is interesting that these values are larger by 10 to 40-fold than the value obtained for fibronectin (Table 2).
Fig. 2. Quantitation of cell spreading on ricin-glass matrices. A. Experiments were similar to those shown in Fig. 1. Cells were assessed as having a round or spread morphology by surveying at least 5 separate fields each containing 50-100 cells as follows: +, < 20% cells spread; 2+, 20-40% cells spread; 3+, 40-60% cells spread; 4+, > 80% cells spread. The ricin concentrations used to derivatize the glass coverslips are indicated. B. The actual amounts of ricin covalently linked to the glass coverslips were estimated using $^{125}$I-ricin as described in the text. These coverslips were then used to promote cell attachment and spreading. After washing in phosphate-buffered saline at least 5 fields containing 50-100 cells were counted for numbers of attached cells and the percentage of cells with a well-spread morphology. (○) BHK wild-type cells (○); (□) Ric$^{14}$ (○); (●) Ric$^{15}$ (○); (×) Ric$^{21}$ cell spreading (○); (▲) Ric$^{21}$ cell attachment. The numbers in parentheses indicate relative ricin-binding sites on each cell line.
Fig. 3. Photomicrographs of cells spreading on concanavalin A–glass matrices. Glass coverslips derivatized with 1 mg ml⁻¹ of concanavalin A were used to promote attachment and spreading of BHK wild-type cells (A), Ric²¹⁴ (B) and Ric²²¹ (C) cells. x 260.
Modulation of BHK cell adhesion

Adhesion of ricin-resistant cells

The results so far show that adhesion of trypsinized BHK cells to protein-derivatized matrices can be modulated by variation of the density of the substratum-attached proteins. We examined next the quantitative relationship between the number of cell-surface receptors for a particular protein and the ability of a matrix containing the protein to mediate cell attachment and spreading.

![Graph](image)

Fig. 4. Quantitation of cell spreading on concanavalin A-glass. The amounts of glass-derivatized concanavalin A were determined using [116]lectin as described in Materials and methods. Trypsinized cells were added and their appearance after incubation at 37 °C for 2 h was assessed as round or spread as before (see Fig. 2). (○) BHK wild-type cells; (x) Ric Ki4; (#) Ric B2i cells.

Ricin-resistant BHK cells, RicR<sub>14</sub>, RicR<sub>15</sub> and RicR<sub>21</sub>, have been studied in detail (Meager, Ungkitchanukit & Hughes, 1976) and found to carry only 10% of the ricin-binding sites present on wild-type BHK cells. Loss of ricin-binding sites is due to deficiencies in specific glycosyl transferases involved in assembly of asparagine-linked oligosaccharides of glycoproteins, including cell-surface glycoproteins (Meager, Ungkitchanukit, Nairn & Hughes, 1975; P. Vischer & Hughes, unpublished results). The ricin-resistant cells become attached and spread on ricin-derivatized glass (Figs. 1, 2). However, the concentration of ricin required for stable cell adhesion and to trigger a change from rounded to well-spread morphology was at least 10 times that required for the wild-type BHK cells (Figs. 1, 2). Thus there appears to be an inverse relationship between the number of cell receptors for ricin in these cells and the minimum concentration of matrix-attached ricin required to promote stable cell adhesion and spreading (Table 2).

The relatively poor ability of ricin-resistant cell lines to adhere to lectin-coated substratum was restricted to ricin and probably other galactose-binding (Baenziger & Fiete, 1979) lectins. The mutant cell lines carry normal amounts of binding sites for concanavalin A (Meager et al. 1975, 1976), a lectin specific for the oligomannosyl core sequence of asparagine-linked oligosaccharides. When the trypsinized mutant cells
were added to glass substrata derivatized with various amounts of concanavalin A, the cells became attached and spread out (Fig. 3) in a manner indistinguishable from the behaviour of wild-type BHK cells (Fig. 4, Table 2).

We have found consistently that cells become attached to lectin-derivatized glass at lectin densities significantly lower than those needed to promote cell spreading. The same seems to be true for fibronectin-mediated cell attachment (our unpublished results). This is illustrated for RicR2i cells interacting with ricin in Fig. 2B, but was observed for all cell lines examined. The relationship between the minimum concentration of ricin required to promote glass attachment of the BHK cell lines and the relative numbers of cell-surface binding sites for ricin was exactly as described above for cell spreading (results not shown).

**DISCUSSION**

The interaction of trypsinized BHK cells with the model substrata described here is clearly a complex sequence of events. As described previously (Grinnell, 1974, 1978; Hughes *et al.* 1979a; Hughes, Mills & Courtois, 1979) for cells spreading on fibronectin or in serum-containing medium, cells must first become attached by binding of cell-surface receptors with substrate-immobilized proteins. These interactions are multiplied, perhaps by recruitment of mobile cell-surface receptors into restricted areas of the cell surface contacting the glass substratum, as suggested by other studies on cell–substratum interactions (Thorn, Cox, Safford & Rees, 1979; Wright & Karnovsky, 1979), and the clustering of surface glycoproteins possibly occurring in intercellular adhesions (Letourneau, 1979; Juliano & Gagalong, 1979). Grinnell (1980) has shown elegantly that the putative receptors for fibronectin are depleted from the top side of cells spread out onto a fibronectin-coated substratum. Provided a minimum number of contacts are made, the cell is then triggered into a morphological transformation with a flattened cytoplasm and an underside containing areas of close apposition to the substratum, as revealed by interference-reflexion microscopy (Heath & Dunn, 1978).

Our major findings in the present study are that the morphological transformation can be modulated by varying either the extracellular matrix density of cell-surface binding proteins or the density of cell-surface receptor glycoproteins interacting with the matrix. The relationship appears to be quantitative in the sense that a reduction of 90% in cell-surface receptors for ricin leads to a 10-fold higher requirement for matrix-bound ricin to trigger cell attachment and spreading. As far as we are aware, this is the first demonstration of the quantitative relationship of binding between complementary molecules to promote stable cell adhesions.

The relative abilities of different lectins to promote adhesion (Table 1), as well as previous data obtained using cells depleted in surface asparagine-linked oligosaccharides (Butters *et al.* 1980), suggest that membrane species that mediate adhesion are not rich in serine or threonine-linked glycans; thus peanut agglutinin, which is likely to bind primarily to asialo-O-glycans, is an inefficient mediator of spreading, while concanavalin A, which binds N-glycans, is much more effective.
A threshold phenomenon in various cell adhesion or aggregation properties as described in this and our previous report (Hughes et al. 1979a) has been observed in other systems (Weigel et al. 1979; Rando & Bangenter, 1979; Rando, Orr & Bangenter, 1979). We do not know at present whether this behaviour reflects simply a requirement for a minimum number of bonds between the underside of the cell and the substratum or if some co-operative interaction involving mobile cell-surface components and matrix-bound proteins is involved. Co-operativity may be implied by the failure of

![Fig. 5. Models of cell adhesion to protein matrices. Trypsinized cells carry many surface glycoproteins binding matrix-attached protein. The cells attach by a critical number of interactions between complementary molecules, and the cell transforms subsequently into a well-spread cell with limited areas of tight contact between cell and substratum (adhesive plaques), a thin cytoplasm and organized cytoskeleton. A matrix-bound protein of broad specificity, e.g. ricin or concanavalin A, mediates cell attachment but much of the binding is non-productive of secondary events in cell spreading and the cells remain poorly spread. By increasing the matrix density of broad specificity proteins the threshold number of binding interactions is exceeded and a stably spread cell is formed.](image)

serum fibronectin to bind from solution to the BHK cell surface (Hughes, unpublished), re-arrangements of the actomyosin system (Thom et al. 1979), and the lag period observed in the adhesion of chick hepatocytes to acrylamide gels carrying concentrations of cell-surface binding substances above the threshold (Weigel et al. 1979).

Taking our results together we propose the following model (Fig. 5). Trypsinized cells retain surface glycoprotein and other receptors that bind to matrix-immobilized protein. In the case of proteins such as fibronectins, the specificity of binding is such that only a sub-set of receptors that may be carbohydrate-based (Pena & Hughes, 1978; Hughes et al. 1980; Butters et al. 1980) can interact. Provided a sufficient number of such interactions are formed the cells become attached and begin to flatten down. In time these cells transform into a well-spread morphology with a thin
cytoplasm and nucleus tightly flattened down to the substratum; and focal adhesions or adhesive plaques between the underside of the cell and the substratum are formed. A less specific matrix-bound protein such as ricin, which binds to 20–30 glycoprotein species of the BHK cell surface (Pearlstein, 1977; our unpublished results), also mediates the passive attachment of cells by interactions with many glycoprotein species, some of which are analogous to those species that interact with fibronectin (Fig. 5c). Our present results imply that, although interactions with non-specific proteins such as ricin and concanavalin A may be as numerous as in cells attached to a fibronectin matrix at equivalent lattice densities, sufficient interactions with specific glycoproteins mediating the morphological transformation of attached cells are not formed. Hence, much higher lattice densities of matrix-attached lectins are needed to satisfy the minimum requirements for active cell spreading.

Other recent results from our laboratory are in support of this general proposal (Aplin, Hughes, C. L. Jaffe & N. Sharon, unpublished data). Using matrix-immobilized proteins containing photo-labile cross-linking reagents, we have been able to probe the underside of well-spread cells and to identify cell-membrane components in closest proximity to the matrix protein. A glycoprotein fraction of molecular weight 47,000 has been isolated, using this technique, from BHK cells spread out onto matrices consisting of fibronectin, ricin, concanavalin A and soybean agglutinin (the latter using neuraminidase-treated cells). This is evidence that fibronectin and the lectins may be rather selective in binding to a particular glycoprotein fraction of the well-spread BHK cell surface. We assume that during the transformation to a well-spread morphology, interactions between general carbohydrate-binding reagents, such as ricin or concanavalin A, and the cell surface sort out so that a particular glycoprotein fraction ends up in the region of closest contact with the substratum. Possibly, binding of ricin and other lectins to this glycoprotein is of significantly higher affinity than binding to other surface glycoproteins irrelevant to the spreading process. This seems unlikely in view of the very different carbohydrate specificities of the lectins we have examined. Alternatively, the binding of matrix-attached proteins to the glycoprotein fraction mediating stable cell spreading may trigger a cellular response to stabilize these contacts at the expense of others. Possibly, secretion of other components, identified in spreading cells at adhesive plaques or in footprints remaining attached to the substratum after removal of cells, such as cellular fibronectin and other proteins (Grinnell & Field, 1979; Culp & Buniel, 1976), proteoglycans or collagen (Culp et al. 1979), is activated and may be one factor. An additional factor may be that re-arrangement of cytoskeletal elements occurs at plasma membrane sites containing the glycoproteins acting productively to promote cell flattening on the various matrices. Rearrangements of cytoplasmic filaments occur in spreading fibroblasts (Gordon & Bushnell, 1979; Badley, Woods, Carruthers & Rees, 1980). Actin-based stress fibres appear to terminate at adhesive plaques (Heath & Dunn, 1978; Wehland, Osborn & Weber, 1979), and a transmembrane connection between extracellular fibronectin fibres and microfilament bundles has been observed (Singer, 1979). α-Actinin (Wehland et al. 1979) and vinculin (Geiger, 1979) have each been suggested to act as anchors for microfilaments at the cytoplasmic face of the plasma membrane, but it is not known at
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present whether these or other cytoskeletal proteins penetrate the membrane to interact with substratum or, more likely, whether the linkage occurs by interaction with transmembrane (glyco)proteins in contact with the substratum.

Our present results showing modulation of cell-substratum adhesions by the densities of either cell-surface receptors or matrix-bound proteins interacting with these receptors may have a bearing on cell motility. Presumably, cells migrating over a substratum break focal adhesions and make fresh ones in the direction of motion. Turnover of surface glycoproteins is a well-established biological property of cells (Warren, 1969) and could contribute to mechanisms involved in cell locomotion. A study of cell locomotory activity on glass-based protein matrices with increasing affinity for the cell surface, and using cells carrying different amounts of cell-surface receptors for the immobilized proteins may throw some light on this problem.

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Modulation of BHK cell adhesion


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