Narrow linear strips of adhesive substratum are powerful inducers of both growth and total focal contact area

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Summary

The stimulus to growth that occurs when cells attach to the substratum can be studied with small adhesive islands. Large numbers of these islands can be grouped together into arrays made up of various sizes, and the response of cells to incubation on these arrays allows the anchorage stimulus to be measured. Past work has shown that single isolated cells can be stimulated to proliferate under these circumstances quite as freely as they do in ordinary cultures, and that the maximum response is given by islands whose size is less than 5000 μm².

This anchorage stimulus might be mediated by the cytoskeleton, which assembles rapidly around the points of attachment to the substratum. One possible approach to testing this hypothesis is to expose cells to islands of different shapes, and to search for common factors among the different arrangements of the cytoskeleton that these different islands cause.

Circular islands induced a relatively disordered arrangement of actin fibres. The fibres were attached at one end to foci of vinculin, which sometimes became arranged in a ring around the margin of the island. Triangular islands showed a more orderly arrangement of actin, in three bands parallel to the sides. In this case, the vinculin accumulated at the apices. Long islands only 3 μm wide could also provide effective attachment for the cells. In this shape the actin accumulated in two bands 2 μm or more apart and up to 5 μm high, and the vinculin similarly collected in parallel interrupted bands along the margins of the island. The number of vinculin foci differed on these three different island shapes, and the total area of vinculin was more than three times greater on long islands than on circles or triangles of the same size.

Despite these differences, all three different shapes of island were capable of inducing up to 100 μm² of vinculin foci in each cell. Round and triangular islands induced this maximum amount of vinculin when their size was 5000 μm². Linear islands induced the same amount when they were only 1000 μm².

The effect of different shapes on total vinculin focal area was paralleled by their effects on growth. All three shapes could support a similar amount of proliferation. Round and triangular islands induced the maximum amount of proliferation when they were 5000 μm² in area, and linear islands when they were only 1000 μm². Similarly, linear islands induced as many cells to undergo DNA synthesis as did circles more than twice as large.

These experiments support the idea that focal contacts might act as transducers of the anchorage stimulus to growth. They show that total focal contact area is consistently and quantitatively related to growth, and suggest that the arrangement of the foci or their number may have less importance. Island substrata of different shapes offer a new approach towards the understanding of the involvement of the cytoskeleton in the mechanism of growth regulation.

Key words: anchorage, adhesion, vinculin, focal contacts, growth regulation.

Introduction

Anchorage dependence is a commonplace of tissue culture, and yet its mechanism remains unknown. The importance of the individual cell and its adhesion to the substratum is shown by experiments in which spreading is prevented so that intercellular interactions cannot occur (Folkman and Moscona, 1978; Ben-Ze'ev et al.)
1980). It is known that adhesion occurs at focal contacts, which begin to appear within the first few minutes of the cell arriving on the substratum (Abercrombie et al. 1971; Heaysman and Pegrum, 1982). There is evidence that focal contacts are transducers of signals for growth from the external matrix to the cell interior (Couchman and Rees, 1979; Burridge et al. 1987; Geiger, 1989). Focal contacts can be shown to be sites of localisation of the products of oncogenes such as src (Rohrschneider and Gentry, 1984) and of regulatory enzymes such as protein kinase C (Jaken et al. 1989). They are also easy to detect because of the presence of vinculin on their internal faces (Geiger et al. 1980). However, attachment to the substratum is a stochastic process that happens at different rates in different cells, and this makes the relation between stimulus and response difficult to study.

We have found that anchorage can be titrated quantitatively. Adhesive islands imprinted on a non-adhesive substratum allow precise control of cell shape and eliminate the cell–cell interactions and changes in culture density, which otherwise confuse and modify the response. They have been used to show that the stimulus to activated Swiss 3T3 cells occurs between 500 and 5000 μm² (O'Neill et al. 1986).

Here we report a study of the effect of different shapes of adhesive island on growth and on the actin and vinculin cytoskeleton. The effects of islands on the total area of vinculin foci in each cell closely paralleled their effects on growth. Cells on islands showed a maximum area of vinculin of about 100 μm², which was as much as cells in ordinary cultures. Different shapes of island caused differences in the arrangement of the cytoskeleton, and in the amount of substratum needed to induce a particular amount of vinculin. Narrow strips of substratum 3 μm wide ('linear islands') induced the same total area of vinculin as circles three times larger. Similarly, linear islands could induce the same amount of growth as circles three times larger, and the maximal stimulus to proliferation was given by linear islands with an area of only 1000 μm². Thus, anchorage stimulation was a function of total focal contact area in these experiments.

**Materials and methods**

**Cell culture**

Swiss mouse 3T3 cells were cultured as described before (O'Neill et al. 1986). We used Dulbecco's modification of Eagle's medium supplemented with 10% foetal calf serum, penicillin and streptomycin. Maintenance cultures were dispersed before they reached confluence by washing with a mixture of trypsin and EDTA, and then pipetting in this growth medium. They were renewed from frozen stocks every month. After exposure and development, the dishes were then examined at intervals of 20 min until 50% of the islands were occupied. The medium was then gently agitated and drawn off. They were washed once, covered with 2.0 ml of growth medium and returned to the incubator. They were incubated for 24 h for cytoskeletal studies, 48 h for DNA synthesis studies and 120 h for proliferation studies.

**Suspension cultures**

Cells detached from stock culture dishes were counted in a Coulter electronic particle counter and then diluted to 500 000 cells ml⁻¹. They were then mixed with 9 volumes of a solution of 1.5 % methyl cellulose (Sigma M-0512, 4000 centipoise) in growth medium. We used a positive displacement pipette for mixing, and also to dispense 2.0 ml volumes of this cell suspension into 35 mm dishes.

**Measurement of proliferation**

Final cell numbers were counted after staining their nuclei with bis-benzimide (Hoechst 33258, Sigma Chemical Co.) during the last hour of their lives at a concentration of 50 μg ml⁻¹. The cells were then fixed, mounted in glycerol, and counted in a fluorescence microscope on a hundred islands of each size. Counts were done in duplicate on separate dishes.

**Immunohistochemistry**

DNA synthesis was measured in terms of the number of nuclei containing [³H]thymidine ([³H]dThd) after continuous incubation for 48 h as described previously (O'Neill et al. 1986). We used 2 μCi ml⁻¹ of [³H]dThd at a concentration of 1 μm, which prevented cell division by blocking progress through G2. After incubating, the cells were stained with Hoechst 33258, fixed, coated with a 200 nm film of polyvinylchloride and then finally with autoradiographic stripping film (Kodak AR 10). After exposure and development, the dishes were then examined in a mixture of incident ultraviolet and transmitted visible light to identify islands occupied with single cells, and to count the proportion of thymidine-positive cells on them. Autoradiography is not the only way to detect DNA synthesis in these cells; we obtained similar results with cells incubated with bromodeoxyuridine and reacted with specific labelled antibody (Amersham International, Amersham, UK), but only autoradiography was used in the experiments reported below.

**Immunocytochemical reagents**

We used antibodies to vinculin, fibronectin and vitronectin.
Actin was detected with phalloidin labelled with Texas Red (Sigma P5157). Vinculin was detected with a mouse monoclonal ascites fluid directed against chicken gizzard vinculin (Sigma V-4505, clone VIN-11-5). Vitronectin was detected with antiserum prepared in rabbits directed against bovine vitronectin (CalBiochem 681127), and fibronectin with rabbit antiseraum against human fibronectin (CalBiochem 341640). The binding of antibodies was detected with fluorescein isothiocyanate (FITC)-labelled sheep IgG directed against mouse IgG (Sigma F-6257), FITC-labelled rabbit IgG directed against goat IgG (Sigma F-5880), FITC-labelled goat IgG directed against rabbit IgG (Sigma F-0382) and TRITC-labelled rabbit IgG directed against mouse IgG (Sigma T-2402).

Cytoskeletal staining

Cells were permeabilised and fixed before staining. Island cultures were first washed briefly in ice-cold phosphate-buffered saline. They were then placed on ice and flooded with a cold 0.2% solution of Triton X-100 in Mes saline. Mes saline was buffered with 50 mM 2-(N-morpholino)ethane sulphonic acid (Sigma Chemical Co. no. M-3023) to pH 6.1, and also contained 100 mM KCl, 5 mM MgCl₂, and 3 mM EGTA. After 2 min, the dishes were emptied and an excess of 3.8% formalin, at room temperature, was added. Fixation was allowed to continue for 5 min. Formalin was removed by three washes spread over a period of 30 min.

Suspension cultures were washed free from methyl cellulose by diluting 10-fold in cold saline and centrifuging twice. They were then resuspended in 0.2 ml of saline and diluted tenfold in cold Triton X-100 at a concentration of 0.2%. After 2 min, this was further diluted with a 10-fold excess of 3.8% formalin. After 10 min, the cells were recovered from the formalin by centrifuging, washed twice in saline, and then allowed to settle on a glass microscope slide coated with gelatin hardened in chrome alum (Rogers, 1979). The slide was kept wet so that the cells kept their spherical shape.

Staining for actin and vinculin was done as soon as the cells had been fixed. Antibody directed against vinculin was diluted 1:100 in saline containing 0.5% Triton X-100 and left on the cells for 1 h at room temperature. The cells were washed three times in saline and left soaking in saline for 30 min before substituting FITC-labelled anti-IgG at a dilution of 1:16. After a further 30 min they were washed briefly and then phalloidin labelled with Texas Red at a concentration of 200 ng ml⁻¹ was added. Staining with phalloidin was allowed to continue for 30 min and then the cells were washed in several changes of saline for a minimum of 1 h. They were finally mounted in a mixture of 90% glycerol with 10% phosphate-buffered saline containing paraphenylenediamine at a final concentration of 0.1%. Island cultures were mounted on microscope slides 38 mm wide. All slides were stored at 4°C, and examined within 2 days.

Microscopy

Cells were counted in a conventional fluorescence microscope. Time-lapse cinematography was done on 16 mm photographic film on fields 2.3 mm wide at a temporal magnification of 3000 as described previously (O’Neill et al. 1985). Cytoskeletal observations and measurements of island dimensions were done with a MRC-500 laser scanning confocal microscope attachment.

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(Bio-Rad Microscience, Hemel Hempstead, Herts HP2 7TD). This was mounted on a Nikon Micronphot FX, adapted to isolate the laser beam from the binocular viewing head and equipped for epifluorescence (Nikon UK Ltd, Telford, Salop. TF7 4EW).

Focal contact size was measured with the MRC-500. Fields 100 μm wide were imaged with a NA 1.4 planapochromat objective under standard conditions. This field was large enough to include even the largest circular or triangular islands, but the longer linear islands needed two fields. The margins of the foci were made easier to detect by spatially filtering and scaling the images according to a standard routine; all the images shown here were treated in this way. Each vinculin focus was digitised by hand, using the 'area' function of the MRC-500 complex menu whose operation is illustrated in the right-hand image of Fig. 5 (below). The total area of foci in each of 25 cells was then used to compute a regression line of area on island size. This process involved between 1000 and 2000 area measurements; we found that a dedicated image analysis system (Kontron IB AS) could measure all the foci in a single cell simultaneously, and at the same time perform a wide range of analyses on their shape, but did not use it for the measurements reported below. Quantitative measurements of antibody binding were made with the same area function.

Results

Behaviour of cells on islands

Islands bound vitronectin nearly as powerfully as glass. Immunofluorescence measurements indicated that the thin palladium film, which had an absorbance of less than 0.05, was a powerful adsorbent of both vitronectin and fibronectin. The polyHEMA underlayer did not adsorb at all. We compared the binding of these surfaces in the microscope after reacting with specific and labelled antibody. If the islands had been exposed to serum-containing medium, they gave a brilliant image with antibody to vitronectin. Measured in arbitrary units, the brightness of this image was 115; glass under the same conditions gave 160, and the surrounding polyHEMA was 8. Omission of either the serum or the specific antibody reduced all the figures to less than 10. If the islands were treated with fibronectin, similar results were obtained when binding was detected with the appropriate specific antibody.

Time-lapse cinematography showed that cells attached and spread normally on islands, as the affinity of islands for vitronectin would suggest. The cells never migrated even briefly beyond the boundaries of the islands, and did not extend filopods over the surrounding polyHEMA to a distance of more than 5 μm. The resolution of the 4X objective used for time-lapse was not sufficient to detect the thinnest filopods, but we found none longer than 12 μm when the cells were imaged confocally in vivo with a NA 1.4 objective after they had been injected with Lucifer Yellow (1%). Since the distance between the islands was always more than 70 μm, the cells can never have come into contact with one another.

Cells spread to a mean maximum area of about 2400 μm² on circular islands. Cells on islands larger than this were free to change their outlines, and rapidly moved about if they were in the presence of growth medium.

Cells on triangular islands responded similarly, and did not differ significantly in the maximum area they reached.

Cells on linear islands were extended into narrow shapes, which reached a minimum width of slightly less than 1 μm. This minimum was always located between the perinuclear region and the extremities; the perinuclear region was not normally less than 7 μm in width and 5 μm in height. The extremities normally covered the full width of the island, that is to say, 3 μm. The mean maximum length reached by cells on linear islands was about 240 μm. On islands of less than maximum length, the cells spread out to cover the whole island, and eventually overlapped its boundaries to adopt a flattened ellipsoidal shape.

Cells on all these islands showed some signs of motility in so far as their shapes changed with time, if serum was present in the medium in normal concentration (10%). Motility was particularly easy to follow on linear islands because the perinuclear region moved back and forth conspicuously. We found that it was possible to follow this movement with a digitiser, and so to measure it as mean speed of translocation of this part of the cell. Fig. 2 shows that this measure of motility bore a smooth relation to island size, and was detectable at 100 μm². It reached a maximum of about 12 μm h⁻¹ on islands 300 μm long.

Effects of islands on the cytoskeleton

We studied the cytoskeleton of cells on these various shapes of island after 24 h of incubation. Attachment to all these islands induced the formation of stress fibres and vinculin foci. These were quite absent from cells incubated in suspension, and suspended cells showed no more than a diffuse low concentration of Triton-insoluble actin in the cortical region. In attached cells, all the stress fibres were attached to vinculin foci at one end, and none of them were ever more than 5 μm from the substratum. Cells on the smaller islands had rounded shapes, which
could be as much as 15 μm high; the upper regions of these cells were as empty of structure as suspended cells.

The actin in circular islands was relatively disordered. Some of it accumulated around the margin in a circular arrangement (left-hand image of Fig. 3). Triangular islands showed a more regular pattern, with most fibrils running parallel to the cell margins to end in fan-shaped arrays at the apices (also shown in Fig. 3). There were also some fine fibrils lying under and over the perinuclear region. Linear islands showed conspicuous parallel bundles of actin lying along the margins of the cell, and separated by at least 2 μm (right-hand image of Fig. 3). This parallel arrangement was most conspicuous on lines with lengths about 100 μm. We did not measure the amount of actin staining in these various cells, but it clearly increased with island size.

Vinculin on circular islands also showed both circular and disordered arrangements. Cells on circles of about 1000 μm$^2$ tended to accumulate all their vinculin in an interrupted circle at the periphery of the island; the distance between these foci was about 2 μm (left-hand image of Fig. 4). Cells on triangles showed a more orderly arrangement with the vinculin accumulating at the apices (also shown in Fig. 4). These foci were relatively large, with a maximum size of about 10 μm$^2$. Vinculin in cells on linear islands was arranged along the

Fig. 3. Distribution of actin in cells on different shapes of island. Actin distributions revealed by fluorescent phalloidin. The circle is 80 μm wide, the triangle has a side of 75 μm, and the line is 60 μm long and 3 μm wide.

Fig. 4. Distribution of vinculin on different shapes of island. Antibody to vinculin revealed by indirect immunofluorescence. Here the circle is 57 μm wide, and the line is 105 μm long. The triangle is the same cell shown in Fig. 3, with a side 75 μm long.

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margins in two parallel interrupted lines, separated by a distance of 2 μm (right-hand image of Fig. 4). These foci were small and quite uniform, with a width of about 0.5 μm, and somewhat more variable length (Fig. 5).

Simultaneous imaging of actin and vinculin signals allowed focal contacts to be identified with confidence. Small isolated flecks of fluorescent immunoglobulin occurred occasionally on the background, but never in the interior of the cell. We found that even areas of vinculin stain as small as 0.1 μm² had a clear connection with actin fibrils, and consequently may represent true focal contacts.

Quantification of vinculin foci
We measured the size distribution, number and total area of vinculin on these different island shapes. Circles supported larger foci as the islands became larger, and ultimately the size distribution reached a maximum of 6 μm² (Fig. 6). The distribution on triangles was similar, but the maximum size on the largest triangles was as great as 10 μm². In contrast, lines only supported small foci, with a narrow size range that was not affected by the size of the island (not shown). Numbers of foci also increased with island size. This was true of all the three shapes, but the absolute numbers differed; most foci were seen on linear islands.

We measured the total focal contact area in cells on all these shapes of island. All were capable of inducing more than 100 μm² of foci. There was some scatter in the measurements, because only 25 cells were measured. It was nevertheless possible to derive regression lines relating total focal area to island size that were reproducible from one experiment to another.

Linear islands induced the same area of focal contacts as circular islands four times larger (Fig. 7). The size ranges we have chosen, which relate to the maximum these cells can spread on the different shapes, proved also to be the appropriate size ranges for the induction of focal contacts. The minimum on the smallest islands of each shape was about 10 μm², and the maximum ranged between 100 and 130 μm². The regression lines showed clear dose–response relationships that were equal in slope but differed in island size by a factor of about 4.

Triangular islands induced the same area of focal contacts as did circles of equal area (not shown). It was not possible to detect any difference between triangles and circles in the total area of focal contacts they induced, or in the relation between total focal area and island size.

These findings were not affected by serum concen-
Fig. 7. Focal contact area on different sizes of island. Total area of the focal contacts in individual cells was counted, and plotted against the size of the island. Regression lines were then calculated to estimate the effect of island size on focal area. (□) linear islands; (■) circular islands.

Fig. 8. Effect of serum starvation on focal contact area. A similar experiment to that described in Fig. 7, but with the serum concentration in the medium reduced to 0.5%. Symbols as before (see Fig. 7).

Fig. 9. Proliferation of cells on different shapes of island. Cells were incubated on island patterns for 5 days, and then the mean number of cells was counted on 100 islands of each size. In a control experiment, the number of cells after incubation for only 3 h was also counted. Data points have been joined to form a curve by interpolation (Stineman, 1980). Square symbols: linear islands; round symbols: circular islands; filled symbols: 5-day incubation; open symbols: 3-h incubation.

Quantification of proliferation
We determined the ability of these different shapes to support proliferation. All of them were able to support a similar increase in cell number, sufficient to produce a mean of seven or more cells on the largest islands. Counts of cell number were quite precise because more than 1000 cells could be included in each titration. There was a very clear difference between the amount of growth on lines and circles of the same size (Fig. 9). Lines were able to support the growth of as many cells as were circles more than three times larger.

Differences in initial seeding number could not account for this difference between circles and lines. Islands were seeded at random, and the number each island captured was dependent on its size; as a consequence the number of cells initially present on circles was in general slightly greater than on lines (also shown in Fig. 9). Greater initial numbers would not be expected to result in smaller final numbers.

Measurements of DNA synthesis confirmed these results. Both lines and circles induced up to 95% of the cells to incorporate acid-insoluble thymidine into their nuclei. However, lines were more efficient, and supported as much DNA synthesis as circles of twice their size (Fig. 10). These measurements also support the idea, suggested by the previous experiment, that circular islands of the largest size are able to support rather more growth than the largest size of lines. It seems possible that the dose–response curve for lines extends a little further than we have measured it, perhaps to 1500 μm².

Triangles were able to support no more DNA synthesis than circles. A measurement of triangles, done at the same time as the previous experiment, showed a near identity with circles (Fig. 11). Other experiments gave similar results. We also determined the effect of lower concentrations of serum (3%) and shorter incubation times (24 h). Lines were rather more sensitive to this moderate serum deprivation when incubated for only...
Island size (/*m²)

Fig. 10. DNA synthesis on different shapes of island. Cells were incubated on island patterns for 2 days, and then the mean proportion that had incorporated thymidine from the medium during this time was counted on 100 islands of each size. Data points have been joined to form a curve by interpolation as before. (□) Linear islands; (●) circular islands.

Relation between foci and proliferation

These results suggest the possibility that there is a strict quantitative relationship between the total area of foci and the amount of proliferation subsequently induced. We have explored this possibility by plotting total focal area against increase in cell number. Focal area was derived by interpolation from the regression lines illustrated in Fig. 7. The data for cell number were obtained from the experiment illustrated in Fig. 9. When these

Discussion

These experiments show that equal anchorage stimulation can be produced by islands of widely different shapes. Even linear strips as narrow as 3 μm could stimulate proliferation as much as unconfined substrata. The size of island required to give this stimulus was different in different shapes, and was as much as three times greater in circles as in lines. Various aspects of the arrangement of the cytoskeleton were different in all three shapes, and only the total amount of focal contacts bore a constant relationship to the stimulus to growth. In all three shapes, the maximum proliferative stimulus was reached by islands of a size sufficient to induce between 80 and 120 μm² of focal contacts. Thus, the stimulus was a function of total focal contact area. This finding is consistent with the idea that focal contacts act as transducers of the anchorage stimulus.

The relation between focal contacts and proliferation must be dynamic. Thus, at least an equal quantity of focal contacts was found in quiescent cells, and serum starvation did not alter their arrangement even though growth had ceased. However, motility could be measured on linear islands. It was only seen if the amount of serum was adequate for growth, and gave a similar dose-response curve to the amount of growth seen. Similarly, focal contacts are known to have a limited lifespan, and this has been measured as about 30 min on islands during active proliferation (Ireland et al. 1989). It therefore seems likely that the importance of total focal contact area lies in its ability to determine the total rate of turnover.

This sort of dynamic study of focal contact lifetime is now more accessible. Recent improvements in the methodology of interference reflection microscopy allow prolonged observation without cell damage (Zand and Albrecht-Buehler, 1989), and even early events in focal

Fig. 11. DNA synthesis on triangular and circular islands. The same experiment as shown in Fig. 10, in this case comparing triangular and circular islands. Both the shapes have stimulated the same amount of DNA synthesis. (△) Triangular islands; (●) circular islands.

24 h, but still showed clearly greater ability to support growth than circles of the same size.

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contact formation have now been observed (Izzard, 1988). Use of these methods on different shapes of island substratum may help to identify the critical events relating focal contact turnover to the proliferative stimulus.

Other workers have found that there are rapid alterations in vinculin and actin distributions in Balb/c 3T3 cells when they are stimulated to grow by PDGF (Hermann and Pledger, 1985). These workers observed dissociation of vinculin during the first hour of stimulus. We have not investigated the early events in anchorage because of the stochastic variability of cells, which demands that larger numbers be studied than our methods can at present handle, but it is evidently possible that dissociation might be the first event when a turnover process is begun. Our findings are certainly in accord with the observation that vinculin synthesis is proportional to cell spreading (Ungar et al. 1986). They may also be related to the enhanced vinculin synthesis seen in migrating squamous epithelium (Zieske et al. 1989).

The effectiveness of linear islands seen in the present experiments might be related to the possibility of inducing tension in different cell shapes. There is evidence that physical stretching of the cell substratum can stimulate growth (Curti9 and Sehar, 1978; DeWitt et al. 1984). Conversely, focal contacts are known to exert tension on the substratum. We have confirmed that this is also true of island substrata by using thick HEMA coats, which give reduced support to palladium films although observations by interference reflection can continue. Under these circumstances, the palladium film can be seen to yield and crumple in the regions underlying focal contacts in a way that can only be explained by the exertion of a local force (unpublished). Consequently, total focal contact area might determine the total tension exerted on the substratum by the cell. This hypothesis is open to experimental test. In particular, it might be possible to measure tension as a function of the distortion of a thick HEMA coat, its thickness and its shear modulus.

A transducing role for focal contacts is consistent with what else is known about the anchorage stimulus. In particular, rate of interchange of solutes between the cell and the bulk medium may be significantly increased when the cell spreads, but cannot account for all the stimulus observed. Evidence for the importance of solute interchange comes from the enhancement of growth by stirring (Stoker, 1973), the observation that this enhancement follows the direction of flow in the medium (Dunn and Ireland, 1987), and that density inhibition causes a 10-fold increase in the local concentration of hydrogen ions (Akatov et al. 1985). In addition, the surface-volume ratio of some cell types is closely related to anchorage stimulation when shape is altered by serum concentration (O'Neill et al. 1986). However, changes in surface-volume ratio can be avoided by studying the responses of cells that have attached to the substratum but have been forced to remain rounded. Under these circumstances, part of the membrane is occluded, and the surface-volume ratio is actually less than in free suspension (O'Neill et al. 1986). This sort of limited anchorage can still stimulate (Folkman and Moscona, 1978) and causes both protein synthesis (Ben-Ze'ev et al. 1980) and DNA synthesis (O'Neill et al. 1986).

The orderly arrangements of actin and vinculin seen on these islands show that strict rules must govern their assembly. In the most general terms, foci seem to inhibit one another. Thus, foci tend to accumulate at the peripheries of circles, the apices of triangles and the margins of lines. A more local inhibition is also seen when distances are short, as in the interrupted arrangement that occurs on linear islands. Segel et al. (1983) have developed a detailed hypothesis for the local spatial periodicity of focal contacts that occurs transiently during the first hour of anchorage, based on the coefficients of lateral diffusion of proteins within the membrane. Our observations show that this periodicity is not always transient, and will allow this hypothesis to be put on a more quantitative basis. The importance of membrane diffusion coefficients in anchorage stimulation is emphasised by the recent finding that the global rate of lateral diffusion of membrane lectin receptors is severely reduced by anchorage (Swaisgood and Schindler, 1989).

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References


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