*Proc. Natl. Acad. Sci. USA*
Vol. 88, pp. 7849–7853, September 1991
Cell Biology

# Insoluble fibronectin activates the Na/H antiporter by clustering and immobilizing integrin $\alpha_5\beta_1$, independent of cell shape

MARTIN ALEXANDER SCHWARTZ*†, CLAUDE LECHENE*‡, AND DONALD E. INGBER§

*Department of Cellular and Molecular Physiology, Harvard Medical School, Boston, MA 02115; ‡Department of Medicine, Brigham and Women's Hospital, Boston, MA 02115; and §Departments of Pathology, Harvard Medical School and Brigham and Women's Hospital, and Department of Surgery, Children's Hospital, Boston, MA 02115

*Communicated by Harden M. McConnell, June 7, 1991*

**ABSTRACT** **Growth of anchorage-dependent cells requires both soluble mitogens and insoluble extracellular matrix molecules such as fibronectin. Soluble growth factors activate chemical signaling pathways and stimulate proliferation by binding to transmembrane receptors. Insoluble fibronectin also binds to cell-surface receptors; however, it is thought to act primarily via effects on the cytoskeleton and cell shape. We recently demonstrated that cell spreading on surface-adsorbed fibronectin activates the Na/H antiporter and that inhibition of this chemical-signaling pathway suppresses growth. We now show that insoluble fibronectin activates the Na/H antiporter by clustering and immobilizing integrin $\alpha_5\beta_1$, independent of effects on cell shape. These results show that an extracellular matrix receptor can behave similarly to a growth factor receptor to activate a signaling pathway implicated in growth control.**

Extracellular matrix (ECM) proteins such as fibronectin are potent regulators of cell growth, differentiation, and gene expression, yet very little is known about the molecular events that mediate these effects. Previous work from our laboratories has demonstrated that spreading of normal fibroblasts (1, 2) and bovine capillary endothelial (BCE) cells (3) leads to rapid activation of the Na/H antiporter and elevation of intracellular pH ($pH_i$). A rise of $pH_i$ resulting from activation of the antiporter is a common early effect of virtually all peptide growth factors examined (4). Increased Na/H exchange appears to be required for growth of BCE cells (3), human umbilical vein endothelial cells (5), C3H 10T½ fibroblasts (data not shown), and macrophages (6) in the presence of bicarbonate. Furthermore, anchorage-independent growth of tumor cells correlates strongly with their ability to maintain an alkaline $pH_i$ independent of adhesion to ECM (7), and transfecting cells with a proton pump, which artificially elevates $pH_i$, was sufficient to induce anchorage-independent growth (8, 9). These results all support a role for the $pH_i$ in control of cell growth by adhesion.

Work in the cell adhesion field has generally accepted the assumption that the effects of adhesion molecules such as fibronectin on growth and differentiation are exerted via effects on cell shape and the cytoskeleton (10, 11). Indeed, the effect of fibronectin on $pH_i$ closely parallels the increase in cell spreading when suspended cells are plated on fibronectin-coated plastic (1, 3). This effect also correlates with cell spreading when cells are plated on surfaces of different adhesivity on which cells spread to different extents (1, 3). Cell spreading, however, is a complex phenomenon. When cells spread, integrin receptors become occupied and clustered at sites of adhesion; intracellular proteins such as talin and vinculin become localized to sites of adhesion; cell shape, nuclear shape, and cytoskeletal organization change; and the balance of mechanical forces within the cytoskeleton and between the cell and the substratum is altered. The aim of the work presented here was to investigate the mechanism by which cell spreading induces activation of the antiporter.

## MATERIALS AND METHODS

**Reagents.** Fibronectin was purified from human plasma by affinity chromatography as described (12). ConA, polylysine, acetylated low density lipoprotein, and goat anti-mouse IgG were purchased from Sigma. Monoclonal antibodies A11B2 to the integrin $\alpha_5$ chain and B1E5 to the integrin $\beta_1$ chain (13) were from Caroline Damsky (University of California, San Francisco). Monoclonal antibody TS 2/16 to the integrin $\beta_1$ chain was from Martin Hemler (Dana–Farber Cancer Institute, Boston).

**Cells.** Mouse C3H 10T½ fibroblasts were grown in Dulbecco's modified Eagle's medium (DMEM)/10% calf serum under 5% $CO_2$. For $pH_i$ experiments, cells were plated at low density in 35-mm dishes in the same medium plus 20 mM Hepes. Some dishes were coated with a high concentration of the nonadhesive polymer, poly(hydroxyethyl methacrylate) (polyHEMA), as described (1). Cells plated on the polyHEMA adhered very weakly and remained entirely round. In some cases, polyHEMA-coated dishes were scraped with a sterile plastic pipet tip to obtain small areas of bare tissue culture plastic, where cells spread normally.

BCE cells were cultured on gelatin-coated plastic in DMEM/10% calf serum/2 mM glutamine/streptomycin and penicillin each at 100 units per ml and endothelial cell mitogen at 10 μg/ml, as described (3). For $pH_i$ experiments, cells were first starved for 48 hr in DMEM/1% calf serum, then plated in 35-mm bacterial dishes in defined, serum-free medium/20 mM Hepes/transferrin at 5 μg/ml/bovine serum albumin at 10 mg/ml/high-density lipoproteins at 10 μg/ml/basic fibroblast growth factor at 2 ng/ml (3). The 35-mm dishes were coated with various adhesive proteins, then blocked with bovine serum albumin at 10 mg/ml, as described (3). BCE cells do not adhere or spread at all in the absence of adhesive proteins under these conditions. In some cases, single 35-mm dishes contained small areas coated with different adhesive proteins. Both BCE and C3H 3T10½ cells were used for $pH_i$ and DNA synthesis measurements 1 day after plating.

**$pH_i$ Measurements.** Measurements of $pH_i$ in single cells were made as described (1, 2). Briefly, cells were loaded with the fluorescent dye 2′,7′-bis(2-carboxyethyl)-5(and 6)-carboxyfluorescein by incubation with the acetoxy-methyl ester at 1–3 μM. Cells were illuminated first with light of 450 nm and then with light of 495 nm. The emitted light at >530

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

Abbreviations: BCE, bovine capillary endothelial cells; $pH_i$, intracellular pH; DMEM, Dubecco's modified Eagle's medium; ECM, extracellular matrix; polyHEMA, poly(hydroxyethyl methacrylate).

†To whom reprint requests should be sent at present address: Committee on Vascular Biology, Research Institute at Scripps Clinic, 10666 North Torrey Pines Road, La Jolla, CA 92037.

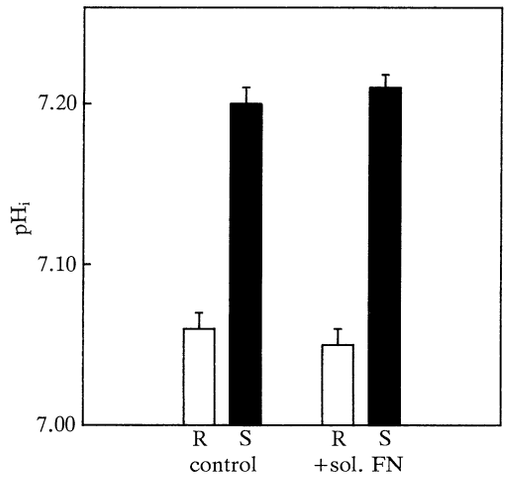


FIG. 1. Effect of soluble fibronectin (sol. FN) on $pH_i$. Spread 10T½ cells on tissue culture plastic (S) or round cells on plastic coated with polyHEMA (R) were incubated in medium with or without fibronectin at 1 mg/ml. Four experiments gave essentially identical results, and similar results were obtained in BCE cells (data not shown). Data are for 1 hr of incubation, but identical results were obtained at all times examined (2 min to 3 hr).

nm from a single cell was recorded each time with a photometer. Values were corrected for background from nearby cell-free areas, and the 495/450 ratio was calculated. Absolute $pH_i$ was determined by comparison with a calibration curve prepared by equilibrating $pH_i$ with medium pH using high potassium medium containing the ionophore nigericin.

All $pH_i$ measurements were made at 37°C in medium with bicarbonate under 5% $CO_2$. Values are means ± SEs from >15 cells unless otherwise noted.

**Beads.** Tosyl-activated 4.5-μm beads (M450; Robbins Scientific, Mountain View, CA) were covalently derivatized with fibronectin or other adhesive proteins at 0.1 mg/ml and then blocked with bovine serum albumin at 10 mg/ml as described (11). Beads were stored at 4°C in sterile DMEM/bovine serum albumin at 1 mg/ml. To prepare beads coated with monoclonal antibodies, beads derivatized with affinity-purified goat anti-mouse IgG were incubated with saturating amounts of monoclonal antibodies for >30 min. The beads were sedimented, rinsed, and resuspended in DMEM/bovine serum albumin at 1 mg/ml.

**Treatment of Cells.** Beads coated with various adhesive ligands were added to cells in 35-mm dishes without mixing or swirling to ensure gradients of beads within the dish. Cells were incubated as described in the text and then transferred to the microscope stage for $pH_i$ measurements. $pH_i$ was usually measured in cells with various numbers of beads bound in each dish. BCE cells were plated either on high fibronectin (2.5 μg/ml) to give spread cells or on 2.5 ng/ml to give round cells.

For experiments with soluble antibodies, BCE cells were plated on bacterial plastic coated either with a high concentration of fibronectin to give well-spread cells or on plastic coated with basic fibroblastic growth factor to give round cells. Cells presumably attach to basic fibroblastic growth factor via cell-surface heparin sulfate molecules (14) and do not spread, alkalinize, or synthesize DNA (data not shown). This method is much more resistant to detachment during low-temperature incubation and subsequent rinses than low fibronectin but yields similar results with respect to $pH_i$. Cells were incubated on ice with soluble antibodies at 10–25 μg/ml and then were transferred to the microscope stage for $pH_i$ measurements.

**Internalization.** Internalization of TS2/16 anti-$\beta_1$ integrin antibody was measured essentially as described (15). Briefly, purified IgG was iodinated with $^{125}I$ and Iodo-Gen. BCE cells in medium with bovine serum albumin at 10 mg/ml were incubated with the iodinated antibody on ice for 1 hr and then rinsed and warmed to 37°C for the indicated period. The cells were then rinsed twice, and internalized antibody was separated from surface-bound antibody by extraction with cold 0.4 M acetic acid/1 M NaCl. The cells were pelleted through extraction buffer containing 10% sucrose, the tubes were frozen and cut, and the $^{125}I$ in the supernatants and pellets was determined by gamma counting. Specific binding was determined by subtracting counts from duplicate samples where cells were preincubated with excess unlabeled antibody.

Internalization of anti-$\beta_1$ IgG on beads was measured as described. Beads were incubated with BCE cells for the indicated length of time and then fixed with 3.7% formaldehyde. The cells were rinsed with phosphate-buffered saline and then incubated with rhodamine-conjugated anti-mouse IgG to stain those remaining on the surface. Labeled and unlabeled beads were scored by fluorescence and phase-contrast microscopy.

## RESULTS

**Receptor Occupancy.** To determine whether occupancy of fibronectin receptors was sufficient to induce Na/H antiporter activation and elevation of $pH_i$, mouse 10T½ cells were treated with soluble fibronectin. Neither fully spread cells on tissue culture plastic nor round cells on polyHEMA showed any change in $pH_i$ between 2 min and 3 hr after addition of soluble fibronectin at 1 mg/ml (Fig. 1), a concentration sufficient to occupy most of the fibronectin receptors


FIG. 2. Binding of beads by cells. 10T½ cells in polyHEMA-coated plastic 35-mm dishes were incubated with $8 \times 10^5$ 4.5-μm beads coated with bovine serum albumin (*A*) or fibronectin (*B*). After 2 hr, cells were photographed under Hoffman optics. (×320.)

(16). Receptors were indeed occupied because the same concentration of fibronectin significantly slowed spreading of cells plated on fibronectin (data not shown), as previously demonstrated (24). Similarly, saturating doses of the peptide GRGDSP, which binds to fibronectin receptors, also failed to induce alkalinization of round 10T½ cells (data not shown). These results demonstrate that occupancy of fibronectin receptors by soluble ligands is not sufficient to trigger increases in $pH_i$.

**Cell Spreading.** To determine whether large-scale changes of cell shape were necessary for activation of the Na/H antiporter, cells were treated with 4.5-$\mu$m beads coated with fibronectin or other ligands. Binding of fibronectin beads to round, poorly attached cells was rapid and specific (ref. 11; Fig. 2) and led to a dose-dependent increase in $pH_i$ (Fig. 3*a*). Spread cells bound fibronectin beads similarly, but $pH_i$ was initially high, and binding of beads induced no further increase. Control experiments showed that beads do not interfere with $pH_i$ measurements because transferring cells to medium containing high potassium and nigericin, which clamps $pH_i$ by equilibration with medium pH, abolished the effects of fibronectin beads on $pH_i$ (Fig. 3*A*). The time course of alkalinization of round cells was similar to that seen after plating cells on fibronectin-coated plastic (Fig. 3*C*; compare to refs. 1 and 3). The increase in $pH_i$ induced by fibronectin was also abolished by addition of 40 $\mu$M hexamethylene amiloride (HMA) (Fig. 3*B*), a specific inhibitor of the antiporter (17). Significant increases in $pH_i$ were not induced by binding of Con A or polylysine-coated beads to 10T½ cells (Fig. 3*B*) or by polylysine or acetylated low-density lipoprotein beads to BCE cells (Fig. 3*D*), even though these beads bind to cells as well as fibronectin beads do. Fibronectin-coated beads did not, however, induce DNA synthesis in round 10T½ (data not shown) or BCE cells (11), even at high doses. We conclude that the interaction of cells with immobilized fibronectin is sufficient to specifically induce activation of the Na/H antiporter in the absence of cell spreading but that activation of the antiporter is not sufficient for cell growth.

**Integrins.** Fibronectin is a large, complex molecule with the potential to interact with a number of cell-surface molecules (18, 19). To address directly the question of which receptor was responsible for effects on $pH_i$, we examined the effects of beads coated with anti-receptor antibodies. Beads coated

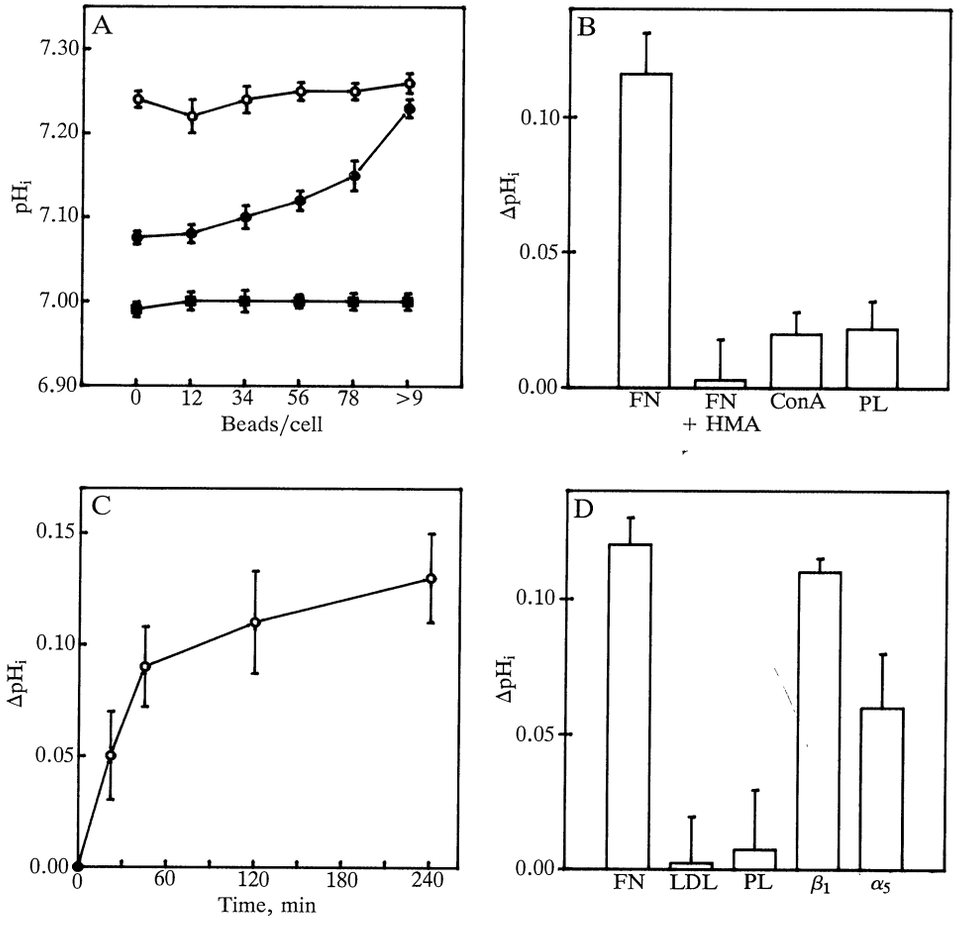


FIG. 3. Effects of beads on $pH_i$. (*A*) Dose dependence for fibronectin beads on 10T½ cells. Spread cells on tissue culture plastic (○) or round cells on polyHEMA (●) were incubated with fibronectin beads for 2 hr. Some round cells were transferred to Hepes-buffered medium without bicarbonate at pH 7.0 with high potassium and nigericin (■). $pH_i$ was determined as a function of the number of beads bound per cell. Three such experiments gave similar results, and similar results were obtained in BCE cells (data not shown). (*B*) Controls. Round 10T½ cells on polyHEMA-coated plastic were incubated 2 hr with beads coated with fibronectin (FN), polylysine (PL), or Con A. $pH_i$ was determined in cells with no beads bound and with more than nine beads bound in the same dish, and the difference between them, $\Delta pH_i$, was calculated. Some cells were incubated for 30 min with 40 $\mu$m of hexamethylene amiloride (HMA) before measuring $pH_i$. (*C*) Time course of alkalinization for fibronectin beads. Fibronectin beads were added to round 10T½ on polyHEMA at time zero. $pH_i$ was measured at subsequent times in >15 cells with bound beads and control cells without beads, and the difference, $\Delta pH_i$, was calculated. (*D*) BCE cells with beads. $pH_i$ was determined in round BCE cells after 2 hr with beads in cells with no beads bound and with more than nine beads bound. Beads were coated with fibronectin (FN), polylysine (PL), acetylated low-density lipoprotein (LDL), antibody A11B2 to the integrin $\beta_1$ chain ($\beta_1$) or antibody B1E5 to the integrin $\alpha_5$ chain ($\alpha_5$). Similar results were obtained with antibody TS2/16 to the $\beta_1$ chain (data not shown).

with antibodies to either the $\alpha_5$ or $\beta_1$ integrin subunits bound specifically to BCE cells and induced significant increases in $pH_i$ (Fig. 3*D*). This alkalinization was also inhibited by hexamethylene amiloride (data not shown), demonstrating that activation of the Na/H antiporter was required. These results strongly suggest that the $\alpha_5\beta_1$ integrin mediates the effects of fibronectin on $pH_i$.

**Receptor Clustering.** We next asked whether local clustering of integrins by fibronectin or antibodies immobilized at high density on beads might be responsible for activation of the antiporter. To address this question, cells were incubated on ice with or without anti-integrin antibodies and then transferred to the heated microscope stage at 37°C. Cells reached 37°C in ≈2 min, after which $pH_i$ was followed in single cells as a function of time. Round BCE cells, treated with monoclonal IgG against the integrin $\beta_1$ chain, showed a transient increase in $pH_i$, which peaked at 10 min and returned to control values by ≈25 min (Fig. 4*A*). Addition of

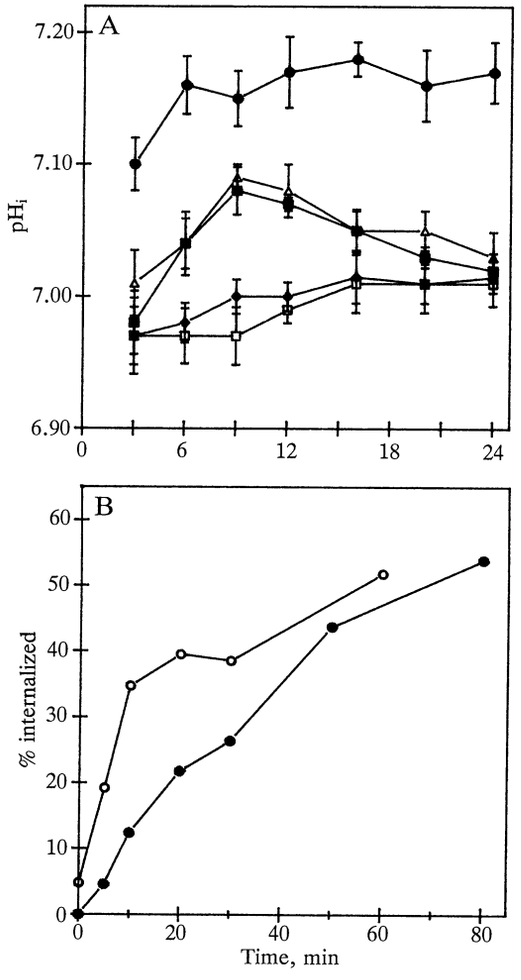


FIG. 4. Soluble antibodies. (*A*) Soluble antibodies and $pH_i$. ●, Spread cells on fibronectin; spread cells gave identical results with or without antibodies. ◆, Round cells on basic fibroblastic growth factor without antibody. ■, Round cells with anti-$\beta_1$ IgG. □, Round cells with anti-$\beta_1$ Fab. △, Round cells incubated with anti-$\beta_1$ Fab for 1 hr, then rinsed and incubated with goat anti-mouse IgG for 30 min. Data points are means ± SE from four single cell time courses. Similar results were obtained for a total of 15–20 cells for each condition. Essentially identical results were obtained with intact antibody A11B2 to the $\beta_1$ chain, although sufficient purified A11B2 IgG was not available for Fab experiments. (*B*) Internalization of integrins. ○, Internalization of soluble TS2/16 anti-$\beta_1$ IgG as a function of time, expressed as a percent of the total specifically bound antibody. ●, Internalization of anti-$\beta_1$ IgG-coated microbeads as a function of time, expressed as percent bound beads.

second antibody against mouse IgG had no additional effect, suggesting that additional clustering was not required. Fully spread cells on a high density of fibronectin had a higher $pH_i$ than round cells at all times, and the time course was identical with or without anti-integrin antibody. The antibody also had a negligible effect on $pH_i$ when simply added to round cells at 37°C (data not shown).

To test the role of integrin clustering more directly, Fab fragments of anti-$\beta_1$ IgG were prepared. Treating round cells with anti-$\beta_1$ Fab had no effect on $pH_i$, but treating cells with anti-$\beta_1$ Fab followed by second antibody again led to a transient increase in $pH_i$, similar to intact IgG. The maximal increase after treatment with IgG or Fab plus second antibody was small (0.08 pH unit in both cases) but was highly significant according to the Student's *t* test. Mean $pH_i$ in IgG-treated cells at 9 min was significantly higher than control cells without antibody ($P < 0.01$) and than the same cells at 24 min ($P < 0.05$). Mean $pH_i$ in cells treated with Fab plus second antibody was higher than control cells ($P < 0.01$) and than the same cells at 24 min ($P < 0.01$.) These results show that local clustering of integrins leads to activation of the Na/H antiporter and, hence, a rise in $pH_i$.

**Receptor Internalization.** The increase in $pH_i$ seen after clustering of receptors with soluble antibodies was transient, unlike the sustained increase in $pH_i$ seen when the same antibodies were immobilized on beads. To determine whether receptor internalization might be responsible for termination of the signal, internalization of $^{125}$I-labeled anti-$\beta_1$ IgG was measured. Internalization was measured by using extraction with acid to distinguish surface-bound from internalized IgG (15). As in the previous experiments, IgG was bound to cells at low temperature, and cells were warmed to 37°C for various lengths of time. Fig. 4*B* shows that 40–50% of the $^{125}$I-labeled IgG was rapidly internalized upon warming. Internalization preceded the drop in $pH_i$ seen in Fig. 4*A*. Similar results on integrin internalization have also been obtained by others (15). Thus, termination of the signal is likely to be due to internalization of a subset of the integrin receptors.

Measurement of internalization of 4.5-μm beads coated with anti-$\beta_1$ IgG revealed, however, that internalization *per se* is not sufficient to terminate antiporter activation. These beads were also engulfed, albeit at a slower rate than soluble antibody (Fig. 4*B*); yet, antiporter activation was maintained for hours. Other work has shown that beads coated with fibronectin or anti-integrin antibodies are phagocytosed but remain associated for many hours with integrins and cytoplasmic proteins, such as talin, that normally associate with the plasma membrane at sites of adhesion (20, 21). Further work will be required to define the fate of internalized integrins and how this fate relates to signaling. It appears likely, however, that integrins internalized on beads remain in a compartment that resembles the plasma membrane and are, therefore, still capable of generating signals.

## DISCUSSION

These results lead to an number of important conclusions regarding the regulation of cell growth by anchorage. (*i*) The proximal event leading to activation of the Na/H antiporter is local clustering of the $\alpha_5\beta_1$ integrin. In this respect, fibronectin therefore resembles peptide growth factors in that both activate chemical signals by inducing clustering of their receptors. This notion is likely to have wider implications. Attachment of cells to immobilized fibronectin induces rapid expression of growth-associated genes (22), and occupancy or clustering of fibronectin receptors by antibodies regulates gene expression and differentiation in other systems (13, 23). Our results suggest that such effects are probably mediated not by the cytoskeleton but by second messengers.

(*ii*) Integrin $\alpha_5\beta_1$ differs from growth factor receptors in that the signal is highly transient when the ligand is not immobilized on a solid surface. This feature may be physiologically relevant because it can explain how cells prevent stimulation by soluble multivalent ECM proteins or complexes.

(*iii*) Control of growth by adhesion to ECM appears to be comprised of at least two components. One is from local interactions at the plasma membranes that result in activation of the Na/H antiporter and perhaps other signaling events. A second component of growth control involves cell spreading, due to the interaction of integrins with both immobilized ECM proteins and with tension-generating cytoskeletal elements. Both chemical signals and changes in cell shape and cytoskeletal organization are required for cell growth. Although past research has focused almost exclusively on interconnections between integrins and the cytoskeleton as a mechanism for signal transduction, our results clearly demonstrated that integrins can act independently of the cytoskeleton to regulate a cytoplasmic second messenger. Thus, further understanding of control of growth and differentiation by ECM will require elucidation not only of how integrins affect the cytoskeleton but also how they regulate chemical signaling pathways inside the cell.

We thank Erin Rupp and Deepanwita Prusty for excellent technical assistance. We thank Caroline Damsky and Martin Hemler for providing the antibodies to integrins. This work was supported by grants from the Lucille B. Markey Charitable Trust, the Elsa U. Pardee foundation (M.A.S.), and the National Institutes of Health (Grant PO1-CA45548 to D.E.I. and Grant 5p441 RR02604 to C.L.).

1. Schwartz, M. A., Both, G. & Lechene, C. (1989) *Proc. Natl. Acad. Sci. USA* **86,** 4525–4529.
2. Schwartz, M. A., Cragoe, E. J. & Lechene, C. P. (1990) *J. Biol. Chem.* **265,** 1327–1332.
3. Ingber, D. E., Prusty, D., Frangioni, J. J., Cragoe, E. J., Lechene, C. P. & Schwartz, M. A. (1990) *J. Cell Biol.* **110,** 1803–1811.
4. Grinstein, S., Rotin, D. & Mason, M. M. (1989) *Biochim. Biophys. Acta* **988,** 73–97.
5. Bussolino, F., Wang, J. M., Turrini, F., Alessi, D., Ghigo, G., Costamagna, C., Pescarmona, G., Mantovani, A. & Bosia, A. (1989) *J. Biol. Chem.* **264,** 18284–18287.
6. Vairo, G., Argyriou, S., Bordun, A. M., Gonda, T. J., Jr., Cragoe, E. J. & Hamilton, J. A. (1990) *J. Biol. Chem.* **265,** 16929–16939.
7. Schwartz, M. A., Rupp, E. E., Frangioni, J. V. & Lechene, C. P. (1990) *Oncogene* **5,** 55–58.
8. Perona, R., Portillo, F., Giraldez, F. & Serrano, R. (1990) *Mol. Cell. Biol.* **10,** 4110–4115.
9. Perona, R. & Serrano, R. (1988) *Nature (London)* **334,** 438–440.
10. Ruoslahti, E. (1988) *Annu. Rev. Biochem.* **57,** 375–413.
11. Ingber, D. E. (1990) *Proc. Natl. Acad. Sci. USA* **87,** 3579–3583.
12. Miekka, S. I., Ingham, K. C. & Menache, D. (1982) *Thromb. Res.* **27,** 1–14.
13. Werb, Z., Tremble, P. M. & Damsky, C. D. (1989) *J. Cell Biol.* **109,** 877–889.
14. Vlodasky, I., Folkman, J., Sullivan, R., Fridman, R., Ishai-Michaeli, R., Sasse, J. & Klagsbrun, M. (1987) *Proc. Natl. Acad. Sci. USA* **84,** 2292–2296.
15. Sczekan, M. M. & Juliano, R. L. (1990) *J. Cell. Physiol.* **142,** 574–580.
16. Hautanen, A., Gailit, J., Mann, D. M. & Ruoslatti, E. (1989) *J. Biol. Chem.* **264,** 1437–1442.
17. Kleyman, T. R. & Cragoe, E. J. (1988) *J. Membr. Biol.* **105,** 1–21.
18. McCarthy, J. B., Skubitz, A. P. N., Zhao, Q., Yi, X., Mickelson, D. J., Klein, D. J. & Furcht, L. T. (1990) *J. Cell Biol.* **110,** 777–787.
19. Hynes, R. O. & Yamada, K. M. (1982) *J. Cell Biol.* **95,** 369–377.
20. Grinnell, F. & Geiger, B. (1986) *Exp. Cell Res.* **162,** 449–461.
21. Wagner, D. D. & Hynes, R. O. (1982) *Exp. Cell Res.* **140,** 373–381.
22. Dike, L. E. & Farmer, S. R. (1988) *Proc. Natl. Acad. Sci. USA* **85,** 6792–6796.
23. Adams, J. C. & Watt, F. M. (1989) *Nature (London)* **340,** 307–309.
24. Yamada, K. M. & Kennedy, D. W. (1984) *J. Cell Biol.* **99,** 29–36.