Control of Intracellular pH and Growth by Fibronectin in Capillary Endothelial Cells

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Abstract. The aim of this work was to analyze the mechanism by which fibronectin (FN) regulates capillary endothelial cell proliferation. Endothelial cell growth can be controlled in chemically-defined medium by varying the density of FN coated on the substratum (Ingber, D. E., and J. Folkman. J. Cell Biol. 1989. 109:317-330). In this system, DNA synthetic rates are stimulated by FN in direct proportion to its effect on cell extension (projected cell areas) both in the presence and absence of saturating amounts of basic FGF. To investigate direct growth signaling by FN, we carried out microfluorometric measurements of intracellular pH (pHi), a cytoplasmic signal that is commonly influenced by soluble mitogens. pHi increased 0.18 pH units as FN coating densities were raised and cells progressed from round to spread. Intracellular alkalinization induced by attachment to FN was rapid and followed the time course of cell spreading. When measured in the presence and absence of FGF, the effects of FN and FGF on pHi were found to be independent and additive. Furthermore, DNA synthesis correlated with pHi, for all combinations of FGF and FN. Ethylisopropylamiloride, a specific inhibitor of the plasma membrane Na+/H+ antiporter, completely suppressed the effects of FN on both pHi and DNA synthesis. However, cytoplasmic pH per se did not appear to be a critical determinant of growth since DNA synthesis was not significantly inhibited when pHi was lowered over the physiological range by varying the pH of the medium. We conclude that FN and FGF exert their growth-modulating effects in part through activation of the Na+/H+ exchanger, although they appear to trigger this system via separate pathways.

Angiogenesis can be triggered by soluble mitogens such as basic FGF (Shing, 1985; Esch et al., 1985). However, insoluble extracellular matrix (ECM) molecules appear to govern whether individual capillary endothelial (CE) cells will either grow or differentiate in response to FGF (Ingber and Folkman, 1989). This regulatory mechanism is critical since successful morphogenesis does not result solely from repeated cell divisions, but also depends on establishment of local growth differentials and formation of differentiated tissue structures at selective sites (Auspurg and Folkman, 1977; Folkman, 1982).

ECM molecules, such as fibronectin (FN), promote cell growth by binding to specific cell surface receptors (Hynes, 1987; Ruoslahti and Pierschbacher, 1987). However, FN differs from peptide growth factors in that it must be presented in an insoluble form (e.g., adsorbed to plastic or organized in a complex ECM) in order for it to influence CE cell proliferation (Ingber, 1990). The growth-promoting effects of insoluble FN also correlate with its ability to promote cell extension (Ingber et al., 1987; Ingber, 1990). This relation is consistent with results of previous studies which demonstrate that anchorage-dependent cells only respond to growth factors by traversing the cell cycle when attached to substrata that can resist cell-generated mechanical loads (Maragoudas, 1973a, b), and thus support cell spreading (Folkman and Moscona, 1978). Yet, little is known about the mechanism by which adhesion to FN and associated changes of cell shape might control cell growth.

In the present study, we investigated whether FN controls CE cell growth in a direct manner, i.e., by modulating intracellular signaling events. For several reasons, we chose to focus on cytoplasmic pH. Intracellular alkalinization, resulting from activation of a transmembrane Na+/H+ antiport on the cell surface, appears to be a common early effect of virtually every growth factor tested, including FGF (L’Allemain et al., 1984; Pouyssegur et al., 1984; Moelenaar, 1986; Moenner et al., 1987; Grinstein et al., 1989). A number of cytoplasmic oncogenes (Doppler et al., 1987; Schwartz et

1. Abbreviations used in this paper: BCECF-AM, 2', 7'-bis-(2-carboxyethyl)-5(and-6)carboxyfluorescein acetoxymethyl ester; CE, capillary endothelial (cells); DM + FGF, defined medium including DME, transferrin (5 μg/ml), HDL (10 μg/ml), 1% BSA, 20 mM Hepes, and FGF (0.2 ng/ml); DM - FGF, defined medium without FGF; ECM, extracellular matrix; EIPA, ethylisopropylamiloride; FN, fibronectin; pHi, intracellular pH.
Materials and Methods

Experimental System

CE cells were isolated from bovine adrenal cortex as previously described (Folkman et al., 1979) and serially passaged on gelatin-coated 6-well tissue culture dishes in DMEM supplemented with 10% calf serum, 2 mM glutamine, 100 μg/ml penicillin, 100 μg/ml streptomycin (Gibco Laboratories, Grand Island, NY), and 10 μg/ml endothelial mitogen (Biomedical Technologies, Inc., Stoughton, MA). Confluent endothelial monolayers were refed with DMEM containing 1% calf serum (without endothelial mitogen) 2 d before the experiment to ensure that the cells were quiescent and that exogenous growth factors were not accidentally carried over into the experimental medium. Quiescent monolayers were then dissociated into single cells by brief exposure (1–2 min) to trypsin-EDTA (Gibco Laboratories) and transferred to DMEM containing 1% BSA (Fraction V; Armour Pharmaceutical Co., Tarrytown, NY). Cell numbers were measured using a counter (Coulter Electronics Inc., Hialeah, FL). Cell aliquots were then pelleted, washed in DMEM containing 1% BSA, and resuspended in defined medium (DM + FGF) consisting of DMEM (with bicarbonate) supplemented with 5 μg/ml transferrin (Collaborative Research, Rockville, MD), 1% BSA, and 2 ng/ml recombinant basic FGF (kindly supplied by Takeda Chemical Industries Limited, Osaka, Japan). Heps buffer, pH 7.4 (Gibco Laboratories) was also included in the defined medium (20 mM, final concentration) in order to minimize variations of medium pH during the course of these experiments.

CE cells were plated in DM + FGF onto FN-coated 35-mm dishes (2 × 10^5 cells/dish) for determination of intracellular pH, morphometry, and [3H]thymidine autoradiography and 96-well plates (5 × 10^3 cells/well) for quantification of DNA synthesis on a per cell basis and morphometric analysis. All experiments used cells that were cultured for 18–24 h at 37°C. Similar studies were also carried out using DM without FGF (DM – FGF) as well as DMEM in the absence of any additional supplements (i.e., without HEPES, transferrin, or BSA).

To analyze the role of the Na+/H+ exchanger, cells previously plated in DM + FGF were transferred to medium composed of 25 mM NaHCO₃, 135 mM choline chloride, 1.8 mM CaCl₂, 1.0 mM MgCl₂, 4.0 mM KCl, MEM amino acids, and 10 mM glucose (Boyardsky et al., 1988), equilibrated with 5% CO₂. Ethylisopropylamiloride (EIPA) was added and pH i was measured either 1 or 24 h later, as described below. To determine the effects of externally modifying pH i, cells were cultured in DM + FGF minus bicarbonate. Medium pH was controlled using 20 mM Hepes buffer of varying pH.

Preparation of FN-coated Substrata

Human FN (Organon Teknika-Cappel, Malvern, PA) was coated onto bacteriological plastic dishes (i.e., not chemically treated for tissue culture) using a high pH adsorption technique as previously described (Ingrber et al., 1987). In brief, FN was diluted in 0.1 M carbonate buffer, pH 9.4, plated on dishes, and allowed to incubate overnight at 4°C. For analysis of effects on cell shape and growth, FN was added to wells of 96-well plates (Immunon II; Dynatech Laboratories Inc., Alexandria, VA) at 5, 25, 50, 100, 250, and 500 ng/well. For experimental determination of pH i, droplets of carbonate buffer containing 10, 25, and 2,500 ng FN/50 μl were plated separately within the same 35-mm dish (Falcon 1008; Becton Dickinson & Co., Lincoln Park, NJ). In this manner, three different FN coating densities were obtained within each culture dish used for pH measurements. Immediately before use, the carbonate coating buffer was removed and dishes were washed sequentially with PBS, DMEM, and DMEM containing 1% BSA.

Cell Growth Determination

To determine CE cell DNA synthetic rates within CE cells, [3H]thymidine (6 Ci/mmol, 5 μCi/ml final concentration; New England Nuclear, Boston, MA) was added to the defined medium, 90 min after cell plating. After 18-h incubation at 37°C, incorporation of [3H]thymidine into TCA-precipitable material was measured directly within adherent cells on FN-coated 96-well plates as previously described (Ingrber et al., 1987). To determine DNA synthesis on a per cell basis, attached CE cells cultured in parallel dishes were trypsinized, pooled, and counted using a counter (Coulter Electronics Inc.). We have previously shown that CE cell DNA synthetic rates correlate directly with increases in cell number (i.e., cell proliferation) using this experimental system (Ingrber, 1990). In experiments with medium of varying pH i, cells were allowed to attach to FN-coated 96-well plates (500 ng/well) in complete DM + FGF. After 4 h, the medium was removed and replaced with bicarbonate-free DM + FGF of varying pH, supplemented with [3H]thymidine. DNA synthesis was measured 18 h later. Similar experiments were also performed by plating cells directly in different pH media; these experiments yielded identical results. Experiments using EIPA were carried out in a similar manner except that the low sodium medium was used. Control levels of DNA synthesis in CE cells cultured in low sodium medium were ~60% of those measured in normal culture medium.

 Autoradiography of [3H]thymidine-labeled cells was carried out using cells grown in 35-mm dishes in parallel with those used for pH measurements. After 24 h of culture in DMEM containing [3H]thymidine (1 μCi/ml), excess nonradioactive thymidine (2 mM final concentration) was added and allowed to equilibrate with cytoplasmic pools for 15 min at 37°C. Cells were then briefly fixed by adding glutaraldehyde directly to the medium (2% final concentration), washed with PBS, and dehydrated in methanol. Autoradiographic grains were developed directly on the surface of the dish using Kodak NTB-2 nuclear track emulsion and D-19 developer. Photographs were taken on a Nikon Diaphot microscope using Hoffman Optics and Kodak Plus-X Pan film.

Cell Shape Determination

Cell shape changes were quantitated by measuring projected cell areas using a modified version of our previously published technique (Ingrber et al., 1987). In brief, cells attached to dishes coated with varying concentrations of FN were fixed with glutaraldehyde, dehydrated in methanol, and stained with Coomasie brilliant blue. Stained cells were visualized on a video monitor using a Zeiss photomicroscope in conjunction with an Amsco video camera. Average projected cell areas were determined by computerized image analysis using an Image Technology image processor in conjunction with an IBM PC 2 Model 30 computer. For each cell shape determination, >30 cells were chosen from six randomly selected areas in three different wells. At least 15 randomly selected cells were used for measurements of the shape of cells used for pH measurements.

Determination of Intracellular pH

The cytoplasmic pH of single CE cells was measured by microfluorimetry as previously described (Schwartz et al., 1989a, 1990). The pH-sensitive fluorochrome, 2',7'-bis-(2-carboxyethyl)-5(and-6)carboxyfluorescein, ace-
Control of CE Cell Growth and Form by FN

CE cells cannot attach to bacteriological dishes in the absence of serum or preadsorbed ECM proteins and therefore are completely dependent upon exogenous FN for attachment and spreading. Under these conditions, CE cell spreading (projected cell areas) and DNA synthetic levels increased in parallel when the density of adsorbed FN attachment sites was raised (Fig. 1, A and B). DNA synthetic rates (normalized for cell number) increased ~100-fold as CE cells progressed from round to maximally spread in chemically defined medium supplemented with a constant, saturating amount of soluble FGF (2 ng/ml).

Control of Cytoplasmic pH by FGF and FN

The effects of FN on pH, were determined in cells grown on small areas coated with different FN densities within the same 35-mm culture dish. This protocol was used to be certain that observed alterations of pH were due to differences in FN-coating density rather than to possible variations in the medium between dishes (e.g., due to differences in cell density, cell death, secretion, or depletion of growth factors, etc.). These studies demonstrated that cytoplasmic pH increased as FN-coating densities were raised, even in the presence of a saturating concentration of FGF (Fig. 2). CE cells grown on the highest FN density were 0.18 units more alkaline than round cells on the low FN concentration. These differences were not due to optical artifacts since spread and round cells had the same cytoplasmic pH when nigericin was used to equilibrate pH, with medium pH, and since the pH, difference was abolished by EIPA (see below).

The next step was to determine whether FN modulated the effects of FGF on pH, or if it regulated pH, independently. Therefore, cytoplasmic pH was measured within cells grown on different FN-coating densities in the absence of FGF. Cells without FGF had a lower pH, on all FN concentrations, however, they displayed a similar FN-dependent increase of cytoplasmic pH (Fig. 2). When FGF was removed from the defined medium, cell spreading was also less exten-

![Figure 1. Effects of surface-adsorbed FN on DNA synthesis (A) and cell spreading (B). CE cells were grown on bacteriological plastic coated with the indicated amount of FN in defined medium in the absence (open circles) or presence (closed circles) of a saturating amount of FGF (2 ng/ml) as described in Materials and Methods.](Image)

![Figure 2. Effects of FN and soluble growth factors on pH. Cytoplasmic pH was measured within cells grown in areas coated with different FN densities within the same 35-mm dish in DM + FGF, DM – FGF, or DME without any additional supplements. pH measurements were carried out as described in Materials and Methods. The solid, stippled, and open bars represent FN-coating concentrations of 2,500, 25, and 10 ng/50 µl droplet, respectively. Standard error was <10% of the mean for all values presented.](Image)
Figure 3. Light microscopic [3H]thymidine autoradiographs of CE cells grown on different FN densities in DME, in the absence of exogenous mitogens. Separate regions of the same 35-mm culture dish were coated with 10 (a), 25 (b), or 2,500 ng (c) FN as described in Materials and Methods. Note the wide range of cell shape control that can be obtained by varying FN coating densities. All photographs were taken under Hoffman optics at the same magnification. Bar, 60 μm.
Figure 4. Relation between pH, and cell spreading. Cells used for Fig. 2 were analyzed by computerized morphometry and the data were replotted as pH vs. average projected cell area. Closed circles, cells grown in DM & FGF; open circles, cells in DM - FGF; open triangles, cells in DME without any additions. pH values represent means ± SEM. The standard error of the projected cell areas was consistently <12% of the mean.

Role of Cell Shape

Morphometric analysis of cells fixed and stained after completion of pH measurements revealed that pH increased as a linear function of projected cell area when averaged for the entire population, for cells in all three media (Fig. 4). It appears that the dependence of pH on cell extension is similar for all conditions, i.e., the three lines have similar slopes (although some saturation may occur at the highest levels of cell spreading or the highest pH values). This analysis shows that the cytoplasmic pH increase due to addition of FGF can be attributed in part to a small increase in cell spreading, but that FGF also produced a significant increase of pH (~0.06 pH units) separate from its effects on cell shape. Note that this is the first demonstration that a potent angiogenic factor, FGF, raises pH, in capillary cells. The finding that FGF stimulated similar increases of pH, in cells on low and high FN densities indicates that functional FGF receptors exist on both round and spread cells. This type of analysis also suggests that the small decrease of pH, produced by removal of the defined medium supplements, HDL and transferrin, was primarily due to a decrease in cell extension.

Role of the Na+/H+ Exchanger

In bicarbonate-containing medium, cells can regulate their pH via HCO₃⁻/Cl⁻ exchangers as well as by the Na⁺/H⁺ antiporter (Bierman et al., 1988). Therefore, it was necessary to determine which transporter was responsible for the differences in cytoplasmic pH induced by changes of FN coating densities. To inactivate HCO₃⁻/Cl⁻ exchangers, cells were transferred to bicarbonate-free medium buffered with Hepes, pH 7.4. Cells underwent rapid alkalinization due to loss of membrane-permeable CO₂, followed by a slower return to steady state pH within 5-10 min (not shown, but see Bierman et al., 1988). Measurements made 1 h after transfer demonstrated that cells on high and low FN densities returned to their original pH (Fig. 5). Thus, bicarbonate was not required to maintain steady-state cytoplasmic pH or to maintain a pH difference between round and spread CE cells. Also, bicarbonate was not required to maintain differences in CE cell DNA synthetic levels on the different FN densities (not shown).

To test whether the Na⁺/H⁺ exchanger is involved in control of pH, cells in medium with bicarbonate were treated with EIPA, an amiloride analogue that is a more specific inhibitor of the Na⁺/H⁺ exchanger (Vigne et al., 1983; Boyarsky et al., 1988). EIPA competes with Na⁺ for binding to the extracellular site of the antiporter. However, because inhibition is competitive, rather high concentrations are required to completely inhibit proton transport in normal culture medium. We therefore used the approach of L’Allemain et al. (1984) and used medium with reduced sodium (choline chloride was added to keep osmotic strength constant). CE
cells in medium with 25 mM sodium had the same pH as cells in normal medium (not shown). When EIPA was added, cytoplasmic pH decreased over 10-15 min and reached a new, lower steady state within 20 min that remained constant for at least an additional 24 h. The difference in cytoplasmic pH between round and spread cells was almost completely eliminated when cells were exposed to 40 μM EIPA (Fig. 6). The effect of EIPA appeared to be specific since it was antagonized by sodium. In culture medium with 140 mM sodium, approximately three times higher concentrations of EIPA were required to reduce the difference in pH between round and spread cells. The ability of EIPA to induce a rapid and persistent decrease of pH in spread cells, in conjunction with the absence of a requirement for bicarbonate, strongly suggests that activation of the Na+/H+ exchanger is responsible for the effect of FN on pH.

Finally, to confirm that there is a link between FN receptor occupancy and Na+/H+ exchanger activation, pH measurements were made during the first hour after cell plating. We found that cells plated on high FN rapidly increased their pH, relative to cells on low FN and that this intracellular alkalinization could be inhibited by EIPA (Fig. 7). Interestingly, cell spreading on the high FN density also progressed over a similar time course whereas cells on low FN remained entirely round throughout the experiment.

**Cytoplasmic pH and Growth**

To determine the relation between DNA synthesis and pH, we estimated DNA synthetic levels within CE cells used for pH measurements based upon their projected cell areas (as shown in Fig. 4). This was done using the relation between DNA synthesis and cell areas described in Fig. 1. This analysis revealed that DNA synthesis and pH correlate for all combinations of FGF and FN (Fig. 8).

Previous studies suggest that cells require an alkaline pH to proliferate (Grinstein, 1988). The decrease in pH observed in cells on low FN densities might therefore, account for growth suppression in round cells. To test this hypothesis, cells were plated on a high FN density in HEPES-buffered medium (without bicarbonate) in which the medium pH was varied from 7.9 to 6.5. Measurements of pH showed that cytoplasmic pH decreased from 7.3 to 6.9 under these conditions (Fig. 9 A). Measurements of [3H]thymidine incorporation within cells cultured in parallel under similar conditions (and normalized for cell number) showed that lower pH was associated with significant inhibition of DNA synthesis (Fig. 9 B), as previously reported with other cell lines (Musgrove et al., 1987). However, when the relation between pH and DNA synthesis obtained by this method is compared with results from cells grown in normal medium on varying FN coating densities (Fig. 8), it is clear
that a small decrease of pH in the physiological range (i.e., from 7.3 to 7.1) can not alone account for the major reduction of DNA synthesis observed in round cells. In contrast, inhibition of Na+/H+ exchange using EIPA resulted in suppression of both intracellular alkalinization (Figs. 6 and 7) and DNA synthesis (Fig. 10). The inhibitory effects of EIPA on DNA synthesis also appeared to be specific since EIPA was much less inhibitory when added in normal, high sodium-containing medium (e.g., 4.1% inhibition at 20 μM EIPA vs. >70% inhibition at the same dose in low sodium medium). Separate experiments showed that lowering pH by changing the pH of the medium gave similar results in both normal and low sodium medium (not shown). These data suggest that activation of the Na+/H+ exchanger may play a role in CE cell growth control distinct from the subsequent changes of steady-state pH, measured by microfluorimetry.

**Discussion**

We recently showed that CE cells can be switched between modes of growth, differentiation, and involution by altering ECM integrity in vitro (Ingber and Folkman, 1989a; Ingber, 1990) and in vivo (Ingber et al., 1986; Ingber and Folkman, 1988). In these studies, the growth-modulating effects of different ECM configurations appeared to be based on their ability to produce alterations of CE cell shape. In the present study, we set out to determine whether adhesion to FN and associated changes of cell shape modulate CE cell growth by altering chemical signaling pathways. We chose to examine pH, because it is a consequence of activation of a variety of growth signaling pathways and has been suggested to act as a direct regulator of cell growth.

**FN Acts Separately from FGF**

In past studies using fibroblasts, we demonstrated that pH increases induced by cell attachment to standard tissue culture plastic correlate with cell shape changes, reverse rapidly during cell detachment, and result from activation of the Na+/H+ exchanger (Schwartz et al., 1989a, 1990). These studies, however, did not determine the relative importance of different serum mitogens or ECM molecules. To address this question more directly in the present study, CE cells were grown in chemically defined medium that was supplemented with a single type of peptide growth factor, FGF, and cell shape was perturbed in a specific manner by varying FN-coating densities on nonadhesive, bacteriological dishes.

The results of these experiments demonstrate that adhesion to FN acts separately from FGF to activate the Na+/H+ exchanger and thereby regulate CE cell growth. This notion is based on data showing that FN activates the Na+/H+ exchanger to a similar degree with or without FGF in CE cells (Figs. 2 and 7). These data also clearly demonstrate that the pH-modulating effects of FGF and adhesion to FN are additive. Thus, FGF apparently activates the Na+/H+ exchanger through a separate signaling pathway. However, it is important to note that with respect to both growth and pH, FN appears to be the more potent regulator.

**Rapid Integration of Signals**

Cells in culture commonly require multiple stimuli, for example, two or more specific growth factors, in order to progress through the cell cycle (Rudland and Jimenez de Asua, 1979). Although little is known about how the cell processes separate signals, it is generally assumed that integration occurs at the level of gene expression (Denhardt et al., 1986; Dynan, 1989). We have found, however, that intracellular alkalinization is induced within minutes after cell attachment to FN and that pH, shows a strong correlation with DNA synthesis for cells stimulated with all combinations of FN and FGF. The fact that pH is a quantitative marker for DNA synthesis under physiological conditions suggests that these separate growth stimuli may in fact converge at an early step during signal transduction. It has also been observed that FGF and insulin, which have synergistic effects on cell growth (Rudland and Jimenez de Asua, 1979), have synergistic effects on pH (Moolenaar et al., 1983).

**Growth Control: Role of the Na+/H+ Exchanger versus pH**

Past studies have demonstrated inhibition of growth by low pH medium (Musgrove et al., 1987) and by inhibitors of Na+/H+ exchange (L’Allemain et al., 1984), but to our knowledge no comparison has been made between these two methods within the same experimental system. We found that when pH was controlled externally that the cytoplasmic pH of spread cells could be lowered to the level of round cells without substantially inhibiting DNA synthesis. On the other hand, studies using EIPA showed that inhibition of Na+/H+ exchange strongly inhibited growth at the same concentrations at which it inhibited cytoplasmic alkalinization, suggesting that activation of the Na+/H+ exchanger itself may be critical for growth control. These paradoxical results would appear to indicate that Na+/H+ exchange plays a role in cell growth control beyond that of regulating steady-state pH. While no direct evidence to support this idea has been published, several authors have made similar proposals. For example, Williams suggested that activation of the Na+/H+ antiporter might trigger growth as a result of local changes of proton concentration directly beneath the cell surface (Williams, 1988). It has also been proposed that Na+/H+ exchange may regulate growth via influx of sodium and stimulation of the Na+/K+ ATPase (Szwergold et al., 1989), and evidence has been presented that Na+ can be critical for

![Figure 10. Effects of EIPA on DNA synthesis. CE cells were handled as described in Fig. 6 except that they were plated in 96-well plates and only a high FN-coating density (500 ng/well) was used. DNA synthesis was measured after 18 h as described in Materials and Methods. Data is presented as percent maximal DNA synthesis ± SEM (i.e., percent [%H]thymidine incorporation exhibited by cells cultured in low sodium medium in the absence of EIPA). Control levels of DNA synthesis in low sodium medium were ~60% of those measured in CE cells grown in normal culture medium.](image-url)
growth apart from its effects on pH, (Burns and Rozenburg, 1984; Panet et al., 1989).

Transmembrane Signaling by FN

It is likely that FN alters cytoplasmic pH as a result of specific binding interactions with a member of the integrin family of receptors. Although this point has not been directly addressed in the present study, we have recently shown that the growth and shape-modulating effects of insoluble FN can be inhibited by addition of low concentrations of soluble RGD-containing peptides to adherent CE cells (Ingber, 1990) and that cell rounding induced by RGD-peptides produces a rapid decrease in pH (Schwartz et al., 1989a). Interestingly, fibrinogen-integrin interactions appear to similarly modulate the function of the plasma membrane Na⁺/H⁺ exchanger in platelets (Banga et al., 1986), although resultant intracellular alkalinization results in cell aggregation rather than growth in these cells. It also may be relevant that integrin clustering has recently been shown to mediate the effects of FN peptides on gene expression in fibroblasts (Werb et al., 1989), however, no effects on growth were reported in that study.

The pH and growth-modulating effects of FN also may be based, in part, upon its ability to support changes of CE cell shape. Our results clearly demonstrate that changing cell–FN contacts produces parallel effects on cell shape, cytoplasmic pH, and growth. This possibility is also supported by the observation that removal of HDL and transferrin from our defined medium resulted in parallel decreases of cell spreading and pH, regardless of the FN density. The biochemical pathway by which cell shape perturbation might alter pH is completely unknown. However, cell spreading is a mechanochimical process which exerts mechanical tension on various cytoskeletal and membrane components (Ingber and Jamieson, 1985; Ingber and Folkman, 1989a). Tension-sensitive membrane channels have been identified (Guharay and Sachs, 1984) and stretch-activated calcium channels are known to exist on endothelial cell surfaces (Lansman et al., 1987). Local tension-dependent changes of membrane curvature in cell–matrix contact zones could also affect the chemical composition of the plasmalemma in these regions (Williams, 1988) or modulate receptor clustering. However, the role of mechanical tension in the control of pH, remains to be determined.

Conclusion

Many past studies on the effects of ECM and cell shape on growth have focused on regulation of cell sensitivity to soluble growth factors (Folkman and Moscona, 1978; Gospodarowicz et al., 1978; Salomon et al., 1981; Ingber et al., 1987). Thus, one of the most important findings of this study is that FN appears to control growth by activating chemical signaling pathways directly. The growth-modulating effects of FN correlate with its ability to promote cell spreading, activate the cell surface Na⁺/H⁺ exchanger, and raise pH. FGF, a potent endothelial mitogen, also produces intracellular alkalinization during growth stimulation, although apparently through a separate transduction pathway. While physiologic alterations of cytoplasmic pH correlate directly with the effects of FGF and FN on DNA synthesis, externally modulated changes of pH, do not. Thus, activation of the Na⁺/H⁺ exchanger may represent a common signaling pathway that ECM molecules and soluble peptide mitogens use to regulate growth. However, in capillary cells, resultant changes of pH alone do not appear to be sufficient to regulate progression through the cell cycle.

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