INTRODUCTION

Many proliferating cells are rendered quiescent in the G0/G1 phase of the cell cycle by serum deprivation but resume cell division with the readdition of serum or target specific mitogens (Rozengurt, 1986). Most non-transformed cells, however, remain quiescent when placed in suspension despite an abundance of soluble growth factors (Benecke et al., 1978), a phenomenon known as anchorage-dependence of cell proliferation (Stoker et al., 1968). This requirement for cell adhesion during cell cycle progression is confined to G1 events as S phase cells will proceed through the remaining portions of the cell cycle in the absence of cell adhesion (Otsuka and Moskowitz, 1975; Han et al., 1993). Although the mechanisms by which adhesion governs cell proliferation are not completely understood, numerous growth factor-regulated G1 events have been shown to be anchorage-dependent (Dike and Farmer, 1988; Ingber et al., 1990a; Guadagno and Assoian, 1991; Farrell Jr and Greene, 1992; Guadagno et al., 1993). In addition, experiments designed to control cell shape have revealed that some proliferation related processes, especially the ability to progress beyond the G1/S boundary, correlate with the extent to which adherent cells can spread (Maroudas, 1973; Folkman and Moscona, 1978; Ingber, 1990b). Interestingly, the control of cell adhesion over growth factor induced cell proliferation is progressively lost with cell transformation as neoplastic cells become anchorage-independent and capable of proliferating in the absence of cell adhesion and cell spreading (Tucker et al., 1981; Wittelsberger et al., 1981; Raz and Ben-Ze’ev, 1982; Kulesh and Greene, 1986; Farrell Jr and Greene, 1992).

A well characterized marker of the G1 phase of the cell cycle is induction of ornithine decarboxylase (ODC), a highly regulated, rate-limiting enzyme in the synthesis of the polyamines, putrescine, spermidine and spermine (Heby, 1981; Pegg, 1988). Upon mitogenic stimulation of non-transformed cells, ODC activity transiently increases during the mid-G1 phase of the cell cycle followed by the late G1 accumulation of intracellular putrescine. Inhibition of ODC leads to a decrease in cellular content of putrescine and spermidine concomitant with cessation of cell proliferation, a process which can be reversed with the administration of exogenous putrescine demonstrating the absolute requirement for polyamines during cell cycle progression. In addition to the regulation of ornithine decarboxylase and the rate of putrescine uptake in anchorage-dependent and anchorage-independent cells. Plating non-transformed IEC-6 epithelial cells at high versus low cell density restricted cell spreading from 900 μm² to approximately 140 μm², blunted the transient induction of ornithine decarboxylase activity from 202 to 32 pmol ¹⁴CO₂/mg protein per hour and reduced the rate of [¹⁴C]putrescine uptake from 46 to 23 pmol/10⁵ cells per hour. The mean spreading area of the cell population was controlled by coating tissue culture dishes with the nonadhesive polymer, polyHEMA. Ornithine decarboxylase activity and putrescine uptake correlated with cell spreading with minimal spreading (263 μm²) corresponding to an 83% decrease in ornithine decarboxylase activity and 51% decrease in the rate of putrescine uptake. Adding the RGD peptide, Gly-Arg-Gly-Glu-Ser-Pro to the medium of sparsely plated cells resulted in rapid reductions in cell spreading concomitant with dose-dependent decreases in ornithine decarboxylase activity and putrescine uptake. Finally, minimizing cell spreading by depriving cells of sub-stratum contact completely abolished serum-induced increases in ornithine decarboxylase and reduced the rate of putrescine uptake by 47%. In contrast to IEC-6 cells, ornithine decarboxylase of neoplastic HTC-116 cells was constitutively expressed with basal and stimulated activity (193 and 982 pmol ¹⁴CO₂/mg protein per hour, respectively) completely independent of cell adhesion. Putrescine uptake, however, was abolished in the absence of cell adhesion. These data suggest that the induction of ornithine decarboxylase activity and the rate of putrescine uptake correlate with spreading of anchorage-dependent IEC-6 cells and that ornithine decarboxylase activity, but not putrescine uptake, appears to be independent of spreading of neoplastic HTC-116 cells.

Key words: epithelial cell, polyamine, putrescine, ornithine decarboxylase, cell adhesion, cell spreading
requirement of cellular polyamines for anchorage-dependent cell proliferation, cell transformation is accompanied by deregulation and constitutive expression ODC, a process which has recently been reported as both essential and sufficient for anchorage-independent cell proliferation (Avuinen et al., 1992; Hölttä et al., 1993; Moshier et al., 1993).

Due to the central role of polyamines in cell proliferation and cell transformation, we examined the effect of cell adhesion and cell spreading on the induction of ODC activity in anchorage-dependent IEC-6 epithelial cells and neoplastic colonic HTC-116 cells which retain epithelial morphology. Since accumulation of intracellular polyamines is essential for cell cycle progression and not ODC per se, we also examined the effects of cell adhesion on the transport system which is responsible for the uptake of putrescine from the extracellular environment (Seiler and Dezeure, 1990). We report that both processes correlate with the degree of cell spreading of anchorage-dependent epithelial cells and that ODC activity, but not putrescine uptake, is independent of cell adhesion of neoplastic cells. These results suggest that cell adhesion plays a role in governing the processes responsible for polyamine accumulation and that a loss of this regulation may play a role in the deregulation of ODC that accompanies cell transformation.

**MATERIALS AND METHODS**

**Materials**

Dulbecco’s modified Eagle’s medium (DMEM), phosphate buffered saline (PBS), pyridoxal 5-phosphate, DL-dithiothreitol, glutaraldehyde, trichloroacetic acid (TCA) and putrescine were obtained from Sigma (St Louis, MO). L-[1-14C]orotic acid (58 mCi/mmol) and L-[1-14C]putrescine dihydrochloride (90.4 mCi/mmol) were purchased from New England Nuclear (Boston, MA). Fetal bovine serum (FBS) was obtained from Hyclone Laboratories (Logan, UT). Poly-hydroxyethylmethacrylate (PolyHEMA) (Hydron, type NCC) was purchased from Peninsula Laboratories, Inc. (Belmont, CA). Fibronecin coated cultureware was purchased from Collaborative Biochemical Products (Bedford, MA). Cells were obtained from the American Type Culture Collection (Rockville, MD).

**Cells and cell culture**

Non-transformed IEC-6 cells (CRL 1592) and neoplastic HTC-116 (CCL 247) cells were cultured in DMEM, pH 7.4, with 5% FBS at 37°C in a 5% CO2 atmosphere. Cells were passaged in 75 cm2 tissue culture dishes under sterile conditions. The ethanol was evaporated slowly over 24 hours at 37°C in an oven which was leveled and stabilized to minimize vibrations.

**Measurement of ornithine decarboxylase activity**

Under all experimental conditions for ODC analysis, cells were rendered quiescent with 24 hour serum deprivation, a time course reported to synchronize 96% of IEC-6 cells in the G0/G1 phase of the cell cycle (Ginty and Seidell, 1989). Quiescent cells were stimulated with 5% FBS for 4 hours, except where noted, prior to cell collection. The cells were harvested by scraping into ice-cold hypotonic assay buffer consisting of 50 mM Tris, pH 7.4, 0.1 mM EDTA, 0.17 mM 23 lauryl ether, 5 mM sodium fluoride, 2 mM DL-dithiothreitol and 0.1 mM pyridoxal 5-phosphate. Cells were lysed with brief sonication and the resulting homogenates centrifuged at 30,000 g for 20 minutes at 4°C. If assays were not performed immediately, the supernatant was stored at −70°C. Ornithine decarboxylase activity was estimated by incubating an aliquot of the cell extract supernatant with 0.1 μCi L-[1-14C]ornithine and measuring the release of 14CO2. The reaction mixture was incubated for 60 minutes at 37°C in a glass tube tightly sealed with a rubber stopper which supported a plastic center well (Kontes Glass, Vineland, NJ). The released 14CO2 was trapped on filter paper positioned in the center well which had been saturated with 20 μl of 2.0 N NaOH. The reaction was terminated by injecting TCA to a final concentration of 10% into the reaction mixture through the rubber stopper and the mixture was given an additional 60 minutes to ensure complete absorption of 14CO2 onto the filter paper. The paper was removed and radioactivity counted by liquid scintillation spectroscopy. The results were corrected for nonenzymatic background and normalized for protein content (Bradford, 1976) using gamma globulin as a standard. Preliminary studies demonstrated that the assay was proportional to the time and protein concentrations used in these experiments and was completely inhibited by treatment of cells with 5 mM α-difluoromethyl ornithine. Results are reported as picomoles of 14CO2 per milligram protein per hour.

**Measurement of putrescine uptake**

Culture medium was replaced with DMEM devoid of FBS 30 minutes prior to uptake measurements. Uptake was initiated with the addition of 1 μM [1-14C]putrescine to the cell medium at 37°C and terminated after 30 minutes incubation, except where noted, by washing the cells with 4°C PBS, pH 7.4, containing 3 mM unlabeled putrescine. Cells were acid precipitated with 10% TCA and an aliquot of the supernatant analyzed by liquid scintillation microscopy. Duplicate dishes were counted for cell number and the results are reported as picomoles of [14C]putrescine uptake per 105 cells per hour. Preliminary studies revealed uptake was linear with time for 2 hours.

**PolyHEMA coated culture dishes**

Altered substrate adherence was accomplished by coating tissue culture dishes with the non-adhesive polymer, polyHEMA as previously described (Folkman and Moscona, 1978). Briefly, 0.1% polyHEMA stock solution was prepared by dissolving the polymer in 95% ethanol overnight. Aliquots of serial dilutions were pipetted onto tissue culture dishes under sterile conditions. The ethanol was evaporated slowly over 24 hours at 37°C in an oven which was leveled and stabilized to minimize vibrations.

**Morphometric analysis**

Cells for light microscopy were gently washed with PBS and photographed under phase contrast. Hemocytometer grids were photographed with each set of micrographs and used for area calculations. The degree of cell spreading was estimated by digitizing the area of the dish covered by individual cells. These 2-D areas were taken from 50 cells from 4 random fields for each experimental condition in which spreading was determined.

Cell shapes were examined with scanning electron microscopy at the extremes of cell spreading using polyHEMA to vary substrate adhesiveness. Thirteen millimeter diameter theranox tissue culture coverslips (Nunc, Inc., Naperville, IL) were placed in the bottom of 35 mm dishes under sterile conditions. PolyHEMA (3×10-5 g/cm2) was added to half of the dishes and dried overnight at 37°C. Cells were plated 2.5×105 cells/cm2 and given 24 hours to allow complete cell attachment. Coverslips with attached cells were gently washed with PBS and fixed for 3 minutes with 2.5% glutaraldehyde-PBS solution. Cells were progressively dehydrated with serial concentrations of ethanol from 10-100%. The dehydration procedure was performed within 9 minutes for longer exposures to ethanol have been reported to dissolve the polyHEMA coating (Watt et al., 1988). Cells were immediately transferred to a critical point dryer using CO2 as an exchange fluid. Coverslips were mounted on stubs and gold sputter coated. Cells were visualized at an incidence angle of 50° with an International Scientific Instrument (ISI-40) scanning electron microscope at a working distance of 15 mm.
RESULTS

Changes in cell density
We initially controlled the spreading of anchorage-dependent IEC-6 cells by plating cells at low (25,000 cell/cm²) and high (200,000 cells/cm²) cell densities, values which approximate 30% confluency and saturation density, respectively. Over 24-36 hours, sparsely plated cells attached and spread extensively on the surface of the culture dish with each cell covering an average of 900 μm² (Fig. 1a). In contrast, high cell density restricted cell spreading to approximately 140 μm² (Fig. 1b). This value is approximated due to the difficulty of discerning the exact cell borders in confluent monolayers. These differences in cell morphology are illustrated in Fig. 1 where it is important to note that each panel represent the same magnification. Under these plating conditions, 24 hour serum deprivation reduced ODC activity to low basal levels (Fig. 2a) confirming that this enzyme is not constitutively expressed in IEC-6 cells. The readdition of 5% FBS to quiescent low density cells resulted in a rapid and transient increase in activity which peaked 4 hours after stimulation at 202 pmol 14CO₂/mg protein per hour and returned to basal levels by 8 hours despite the continued presence of FBS (Fig. 2a). This peak level of activity was not observed at any time point after stimulation of high density cells which were plated and treated under identical conditions (Fig. 2a). In all subsequent experiments, ODC activity was measured at 4 hours following 5% FBS stimulation of cells which had been serum deprived for 24 hours. We also examined the rate of putrescine uptake as a function of cell density, but in contrast to ODC measurements, experiments were conducted 24-36 hours after plating without serum deprivation. The rate of [14C]putrescine uptake by IEC-6 cells plated at high cell density was 50% less than the uptake by sparsely plated cells, decreasing from 46 to 23 pmol/10⁵ cells per hour (Fig. 2b).

Changes in substrate adhesiveness
In addition to a decrease in cell spreading, increased cell density resulted in increased cell-cell contact. The next question we addressed was whether changes in cell spreading could regulate ODC activity and putrescine uptake independent of cell-cell contact. IEC-6 cells were plated on tissue culture dishes with varied degrees of adhesiveness at a low cell density (25,000 cells/cm²) which minimized cell interactions. Altered adhesiveness was produced by coating tissue culture dishes prior to plating with a non-toxic adhesion resistant hydrogel, polyHEMA, as previously described (Folkman and Moscona, 1978). Scanning electron microscopy was employed to reveal three-dimensional cell morphologies 36 hours after plating. Cells attached and spread extensively on untreated

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Fig. 1. Effect of cell density on IEC-6 cell shape. Cells were plated at 25,000 cells/cm² (a) and 200,000 cells/cm² (b) and photographed under phase contrast 36 hours after plating. Both panels represent the same magnification. Bar, 60 μm.

Fig. 2. Effect of cell density on ornithine decarboxylase (ODC) activity and putrescine uptake by IEC-6 cells. Cells were plated at 25,000 cells/cm² (●) and 200,000 cells/cm² (○) and allowed to attach and spread for 24 hours. Cells for ODC analysis (a) were serum starved for an additional 24 hours and collected at sequential time points after the readdition of 5% fetal bovine serum to the cell medium. The rates of [14C]putrescine uptake (b) were determined over sequential time points 36 hours after plating. Values represent means of duplicate determinations. Similar results were obtained in two independent experiments.
culture dishes (Fig. 3a) forming flat cells of various configurations. In contrast, the morphology of cells plated on culture dishes coated with polyHEMA (3 x 10^{-5} g/cm^2) was characterized by a decrease in cell spreading and an increase in cell height (Fig. 3b). Spreading was quantitated using phase contrast microscopy (not shown) by digitizing the two-dimensional area of the dish covered by each cell. Increasing the concentration of polyHEMA (0 to 3.0 x 10^{-5} g/cm^2) in the pre-coating procedure resulted in a progressive decrease in mean cell spreading from 889 to 263 μm^2 (Table 1). Under these conditions, induced ODC activity decreased from 140 to 24 pmol 14CO₂/mg protein per hour and the uptake of [14C]putrescine fell from 37 to 18 pmol/10^5 cells per hour (Table 1).

**Changes in substrate binding**

In the above experiments, cell spreading was restricted from the time of plating for 36-48 hours prior to assay measurements. The next question we addressed was whether the induction of ODC activity and the rate of putrescine uptake would be affected by acute changes in cell spreading. Cell adhesion is mediated by interactions between extracellular matrix proteins (e.g., fibronectin) with cell surface integrins which recognize the amino acid sequence, Arg-Gly-Asp (RGD), of matrix proteins (Ruoslahti and Pierschbacher, 1987). IEC-6 cells, plated in the absence of serum, attached

| Tab 1. Effect of polyHEMA on cell spreading, ornithine decarboxylase (ODC) activity and putrescine uptake in IEC-6 cells |
|---|---|---|
| polyHEMA | Spreading | ODC activity (pmol 14CO₂/mg protein per hour) | [14C]Putrescine uptake (pmol/10^5 cells per hour) |
| 0.0 | 889.1±95.4 | 140.1±22.1 | 36.5±3.1 |
| 0.3 | 618.0±135.5 | 99.1±15.6 | 29.8±3.3 |
| 1.0 | 496.2±98.9 | 77.7±19.8 | 21.4±3.8 |
| 3.0 | 263.1±50.5 | 24.1±14.3 | 17.9±2.7 |

Cells were plated at 25,000 cells/cm^2 on tissue culture dishes coated with various amounts of polyHEMA (3 x 10^{-5} g/cm^2) and allowed to attach and spread for 24 hours. Cell spreading (μm^2) was estimated as described in Materials and Methods. The rate of [14C]putrescine uptake (pmol/10^5 cells per hour) was measured 36 hours after plating or ODC activity (pmol 14CO₂/mg protein per hour) determined at 4 hours following readdition of 5% fetal bovine serum to serum deprived cells. Values are means ± s.d. of 2 independent experiments performed in triplicate.
Cell adhesion and ornithine decarboxylase

and spread on fibronectin coated plates in a fashion similar to that observed on untreated culture dishes (not shown). Thirty six hours after plating, the addition of the peptide Gly-Arg-Gly-Glu-Ser-Pro (GRGDSP) to the cell medium resulted in a decrease in cell spreading which occurred within 2 hours (not shown), presumably by competing for binding with RGD sequences within the matrix coating. Four hours after peptide addition, putrescine uptake was determined or quiescent cells were stimulated for ODC measurements. Additions of GRGDSP peptide from 3.0 μM to 0.3 mM progressively decreased stimulated ODC activity to 46% (Fig. 4a) and putrescine uptake to 72% (Fig. 4b) of values obtained in control cells not receiving no serum supplement. Values represent means ± s.d. of 2 independent experiments performed in duplicate.

Changes in substratum contact

Cell spreading was further investigated by depriving cells of substratum contact. Initially, spread, subconfluent monolayers were serum deprived for 24 hours. For unattached measurements, cells were lifted and seeded onto bacteriological dishes coated with 0.1 mg/cm² polyHEMA to prevent reattachment. Induced ODC activity (hatched bars) was determined 4 hours following the readdition of 5% FBS to the cell medium. Basal ODC activity (open bars) was determined from cells receiving no serum supplement. Values represent means ± s.d. of 2 independent experiments performed in duplicate.

Fig. 5. Effect of cell adhesion on ornithine decarboxylase (ODC) activity in IEC-6 (a) and HTC-116 (b) cells. Subconfluent cell monolayers were serum deprived for 24 hours. For unattached measurements, cells were lifted and seeded onto bacteriological dishes coated with 0.1 mg/cm² polyHEMA to prevent reattachment. Induced ODC activity (hatched bars) was determined 4 hours following the readdition of 5% FBS to the cell medium. Basal ODC activity (open bars) was determined from cells receiving no serum supplement. Values represent means ± s.d. of 2 independent experiments performed in duplicate.

and spread on fibronectin coated plates in a fashion similar to that observed on untreated culture dishes (not shown). Thirty six hours after plating, the addition of the peptide Gly-Arg-Gly-Glu-Ser-Pro (GRGDSP) to the cell medium resulted in a decrease in cell spreading which occurred within 2 hours (not shown), presumably by competing for binding with RGD sequences within the matrix coating. Four hours after peptide addition, putrescine uptake was determined or quiescent cells were stimulated for ODC measurements. Additions of GRGDSP peptide from 3.0 μM to 0.3 mM progressively decreased stimulated ODC activity to 46% (Fig. 4a) and putrescine uptake to 72% (Fig. 4b) of values obtained in control cells not receiving peptide supplements. The peptide Gly-Arg-Gly-Glu-Ser-Pro (GRGESP), with a single amino acid substitution, had no effect on cell spreading (not shown), the induction of ODC activity (Fig. 4a) or putrescine uptake (Fig. 4b).

DISCUSSION

Effect of cell spreading on the induction of ornithine decarboxylase activity

In this study, we utilized four experimental methods to alter cell spreading prior to stimulating quiescent intact cells and subsequently assaying cytosolic extracts for ODC activity. We report that serum-induced increases in ODC activity of anchorage-dependent IEC-6 cells correlates with cell spreading with minimally spread adherent or non-adherent cells unable to induce ODC in response to serum mitogens. Previously, ODC activity of asynchronously dividing IEC-6 cells was reported to decrease as cells approach confluence with activity ultimately reaching non-detectable levels at saturation density (Ginty and
Seidel, 1989). Our data demonstrate that the readaddition of serum to serum-deprived high density cells, 48 hours after plating, results in minimal increases in ODC activity over a time course that encompasses the transient rise and fall in activity of sparsely plated cells. These data suggest that the suppression of ODC was not due to a delay in cell cycle progression nor does the suppression require long term downregulation of gene expression associated with ODC regulation. We also demonstrate that high cell density restricts cell spreading and when cells were held in this configuration independent of cell density, the readaddition of serum did not result in the induction of ODC. Finally, we report that spreading of IEC-6 cells appears to act perrmissively to the actions of growth factors as ODC activity of serum deprived cells remains extremely low regardless of the degree of cell spreading. This notion has previously been postulated with regards to cell proliferation in that the sensitivity of anchorage-dependent cells to humoral factors is reported to be governed by cell spreading (Tucker et al., 1981). Taken together, these findings support the hypothesis that cell spreading plays a role in regulating the mitogenically stimulated mid-G1 induction of ODC activity. In addition these observations suggest that restrictions in cell spreading may play a role in the suppression of ODC activity which accompanies density-dependent inhibition of cell proliferation.

This report also examined the effect of cell adhesion on the induction of ODC activity in malignant cells possessing epithelial morphology. In contrast to the transient expression of activity in sparsely plated IEC-6 cells, we found that ODC was constitutively expressed at high levels in serum-deprived, adherent HTC-116 cells. Notably, the level of ODC expression in these neoplastic cells, in the absence of serum, was equivalent to maximal activity measured for IEC-6 cells in the presence of serum. In addition to the high basal expression, the readaddition of serum resulted in an additional 5-fold increase in ODC activity. Of particular interest, we also report that neither basal nor serum-stimulated ODC activity in HTC-116 cells was dependent on cell adhesion. Other studies have reported that transient ODC activity of non-transformed cells becomes constitutively expressed following viral (Haddox et al., 1980; Hölttä et al., 1993) or oncogene (Sistonen et al., 1987; Hölttä et al., 1988) cell transformations. These and other observations have led to the postulate that during transformation, induction of ODC becomes less responsive to normal control mechanisms and that deregulation of ODC may be a universal feature of neoplastic transformation (Haddox et al., 1980). With recent evidence demonstrating that oncogenicity of ODC (Auvinen et al., 1992; Hölttä et al., 1993), it is conceivable that processes related to cell transformation include a loss in the regulatory mechanisms imparted by cell adhesion, which lead to the deregulation of ODC expression. It should be noted, however, that the data presented in this study compare the effect of cell spreading on the induction of ODC in two distinct cell lines. Further conclusions based on the anchorage-independence of ODC induction should await the examination of these processes during the transformation of a given cell line.

The results of this report on ODC are consistent with what is known about cell spreading and other well-characterized mitogenic G1 events. Stimulating anchorage-dependent 3T3 fibroblasts with platelet derived growth factor (PDGF) results in an early, transient rise in intracellular calcium, a sustained activation of Na+/H+ exchanger which maintains cell alkalinization and an early expression of cellular oncogenes (Rozen- gurtz, 1986). In sparsely plated fibroblasts, the transient increase in cellular calcium peaks and returns to baseline within minutes even in the continued presence of PDGF. In contrast, stimulating cells plated at a higher cell density results in a dramatic decrease in the amplitude of the calcium peak and preventing cell attachment completely suppresses the PDGF response (Tucker et al., 1990). Similarly, our data demonstrate that the transient induction of ODC activity, which peaks 4 hours after stimulation, is diminished at high cell density and abolished in the absence of cell adhesion. Other experiments designed to precisely control cell shape, independent of cell density, indicate that spreading of 3T3 fibroblasts is associated with an increase in intracellular pH via activation of the Na+/H+ exchanger even in the absence of exogenous growth factors (Schwartz, 1989). Similar studies with anchorage-dependent endothelial cells have shown that both basal and fibroblast growth factor stimulated Na+/H+ exchange activity correlate with the degree of cell spreading (Ingber, 1990a). This correlation is consistent with the induction of ODC and cell spreading in our report. Finally, the expression of c-myc and c-ras proto-oncogenes is reported to be dependent on cell spreading in anchorage-dependent fibroblasts and independent of cell spreading in malignant fibroblasts (Farrell Jr and Greene, 1992). Our data confirm this observation in that malignant HTC-116 cells are independent of the regulatory mechanisms imparted by cell adhesion which govern the induction of ODC activity in non-transformed IEC-6 cells. IEC-6 cells, ODC is transiently expressed during mid-G1 phase of the cell cycle (Ginty and Seidel, 1989). Therefore, the effect of cell spreading on the induction of ODC in these cells may be a secondary consequence of cell cycle arrest. While it is well known that cell spreading facilitates the progression of anchorage-dependent cells through the G1-S phase boundary, less is known about the anchorage requirement of early cell cycle events. Recent evidence, however, suggests that certain G1 events can occur independent of cell spreading, suggesting that the initial signaling events remain intact and that cells can exit G0 without cell spreading (Guadagno et al., 1993; Hansen et al., 1994). In view of these observations, the correlation of cell spreading with the mid-G1 induction of ODC activity may be of potential significance in understanding the mechanisms of anchorage-dependent cell cycle progression.

**Effect of cell spreading on the rate of putrescine uptake**

This report also examines the effect of cell spreading on the rate of [14C]putrescine uptake. As with the induction of ODC activity, we report a correlation between cell spreading and putrescine uptake by anchorage-dependent IEC-6 cells. In contrast to the abrogation of ODC activity, however, minimally spread cells retain at least 50% of their capacity to transport putrescine across the plasmalemma with each method of controlling cell shape even in the absence of substratum contact. Uptake of polyamines by growth-arrested cells has previously been reported where putrescine uptake by IEC-6 cells was reduced, but not eliminated, at saturation density (Grollewski et al., 1992). Other studies have reported that increasing cell density results in increased efflux of certain polyamines, particularly spermidine, from the cell (Pegg, 1988) and it is possible that the decrease in uptake with
restricted cell spreading is influenced by an increase in putrescine efflux. This possibility, however, is unlikely. Preliminary experiments indicate that adding putrescine to the cell medium results in an inward flux of putrescine into IEC-6 cells that is essentially unidirectional over 4 hours (not shown). Uptake measurement in this report were conducted over 30 minutes. In addition, high cell density has been reported to not result in putrescine efflux from cultured fibroblasts (DiPasquale et al., 1978).

While putrescine uptake by a few cell types is reportedly enhanced by the presence of growth factors (DiPasquale et al., 1978; Subbaiah and Bagdale, 1982; Gawel-Thompson and Greene, 1989), most cells have a constitutive capacity for polyamine uptake even in the absence of exogenous mitogens. The uptake of putrescine by IEC-6 cells does not appear to be serum modulated (Groblewski et al., 1992). It is important to note that in our report, the effect of cell spreading on putrescine uptake was determined without prior serum deprivation and subsequent stimulation of quiescent cells. Therefore, these data suggest that, unlike the permissive effect of spreading on the serum-induced increases in ODC activity, cell spreading appears to have a direct effect of the uptake of putrescine from the cell medium. Interestingly, this effect does not appear to be lost in anchorage-independent cells as the uptake of [14C]putrescine by malignant HTC-116 cells also decreases in the absence of cell adhesion. Due to the difficulty of controlling cell spreading in these malignant cells, it was not determined whether the anchorage-dependence of putrescine uptake by HTC-116 cells reflects a requirement for cell adhesion or cell spreading.

**Mechanisms linking cell spreading to polyamine synthesis and uptake**

The mechanisms which couple cell spreading to the induction of ornithine decarboxylase activity and the rate of putrescine uptake are not yet defined. There is evidence, however, that mechanisms which govern other growth-related processes are mediated by cell adhesion to extracellular matrix (ECM) molecules via cell surface integrins (Hynes, 1992; Schwartz, 1992; Juliano and Haskill, 1993). Cell adhesion to immobilized ECM molecules results in clustering of integrins and the generation of cellular signals (Schlaepfer et al., 1994; Morino et al., 1995) that are integral to the signal transduction pathways utilized by trophic stimuli. In this context, it is interesting to note that integrin clustering, even in the absence of cell spreading, has been shown to increase intracellular pH via activation of the \( \text{Na}^+\text{H}^+ \) exchanger (Schwartz et al., 1991). Relevant to polyamine metabolism, it is also of interest that a decrease in intracellular pH or the use of a \( \text{Na}^+\text{H}^+ \) exchanger antagonist has been shown to inhibit serum-induced increases in ODC activity (McCormack et al., 1990). It is conceivable, therefore, that the regulation of ODC activity by mitogenic stimulation might be governed directly by integrin clustering or indirectly via sustained activation of earlier mitogenic events required for the enzyme activity. This idea is even more compelling when considering the observation that activities of both \( \text{Na}^+\text{H}^+ \) exchanger and ornithine decarboxylase correlate with anchorage-dependent cell spreading and are constitutively expressed and independent of spreading in transformed cells. This notion would also imply that the effects of cell spreading on ODC activity and putrescine uptake in this report may also be due to changes in integrin clustering that occurs with changes in cell spreading. On the other hand, clustering is not sufficient for DNA synthesis indicating that the ECM regulates entry into the S phase of the cell cycle by modulating cell spreading (Ingber, 1990b). It has been suggested that mechanical signal transduction processes are modulated via alteration in the cytoskeleton (Wang et al., 1993). Changes in actin polymerization associated with either mechanical or chemical signal transduction processes may be involved with the regulation of ODC activity by cell adhesion. In this regard, the cytoskeleton has been proposed as a necessary component in the regulation of ODC activity (Lakshmanan, 1979; Rumsby and Puck, 1982). The cytoskeleton may also be involved in the regulation of putrescine uptake in a manner similar to its possible role in the regulation of stretch activated ion channels within the plasmalemma (Lansman et al., 1987).

The data presented in this study suggest that the induction of ornithine decarboxylase and rate of putrescine uptake correlate with the degree of cell spreading in anchorage-dependent cells. However, since cell spreading and cell adhesion are integrally related, it cannot be determined from these data whether the effects are actually due to changes in cell spreading or cell-matrix interactions. Further investigations, including studies examining the role of the extracellular matrix in modulating polyamine metabolism are needed to elucidate the link between cell spreading and the mechanisms responsible for polyamine accumulation.

**Conclusion**

This study demonstrates a correlation between cell spreading and the induction of ornithine decarboxylase activity and the rate of putrescine uptake by anchorage-dependent IEC-6 cells and that ODC activity, but not putrescine uptake, is independent of cell spreading in neoplastic HTC-116 cells. These results suggest that cell adhesion plays a role in the regulation of mechanisms responsible for polyamine accumulation, especially the mid-G1 induction of ODC activity, and that a loss of this regulation may represent a universal feature of neoplastic transformation.

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