Proc. Natl. Acad. Sci. USA
Vol. 85, pp. 6792–6796, September 1988
Cell Biology

Cell adhesion induces expression of growth-associated genes in suspension-arrested fibroblasts

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Communicated by Sheldon Penman, June 9, 1988

ABSTRACT **A methylcellulose suspension system that prevents cell-surface contact with the substrate was used to study the role of cell adhesion in the regulation of proliferation. The nonadhesive conditions established by suspension culture cause BALB/c 3T3 (A31) cells to enter a G_0 state of growth arrest within 48 hr as defined by an inhibition of DNA synthesis and a suppression of c-myc and histone mRNA expression. The adhesion of these suspension-arrested cells rapidly induces c-fos, c-myc, and actin gene expression. This stimulation did not depend on the presence of serum since the adhesion of suspension-arrested cells, in the absence of serum, also induced the expression of c-fos and c-myc mRNAs. In addition, adhesion onto fibronectin increased the number of cells able to respond to epidermal growth factor and insulin and progress into S phase. These results indicate that adhesion of suspension-arrested cells activates the G_0/G_1 transition independent of growth factors.**

The proliferation of nontransformed fibroblasts in culture is dependent on adhesion to a solid surface. This property is considered a criterion for normal cell growth and is termed anchorage dependence (1). The work of Penman and coworkers (2–4) has revealed that most macromolecular metabolic processes in normal cells are also dependent on adhesion. Suspension of both 3T3 and 3T6 cells in methylcellulose resulted in an inhibition of RNA production and protein synthesis. The recovery of these processes occurs in a coordinate fashion and is dependent on cell adhesion and extensive spreading (5). The stringent regulation of these metabolic processes during suspension and reattachment is lost as cells become increasingly transformed (6, 7).

Adhesion has not been extensively studied as a growth regulatory mechanism. Earlier investigations established a direct correlation between the degree of cell spreading or change in cell shape and growth rate, as measured by DNA synthesis (8–13). Most of our knowledge of the growth cycle, however, has arisen from studies on the action of soluble growth factors on cells arrested by serum deprivation or by contact inhibition. Activation of such cells initiates the transition from G_0 into G_1 and involves a set of second messenger responses that ultimately alter gene expression (14, 15). The specific mechanisms responsible for integrating the effects of growth factors and cell adhesion during cell growth are unknown. Cellular interactions with particular extracellular matrix (ECM) proteins have been suggested as potential components in processes that regulate gene expression associated with growth and differentiation (16, 17). In fact, recent studies have revealed that binding of growth factors during growth activation alters the organization of the cytoskeleton within the adhesion plaque (18).

Studies with the growth control systems of serum deprivation and density inhibition cannot adequately address questions relating to adhesion since both systems are dependent on substrate interactions. We have therefore exploited a system in which cell growth is regulated in response to attachment to a surface. Suspension of BALB/c 3T3 (clone A31-CL7) fibroblasts in methylcellulose culture for prolonged periods of time caused the majority of cells to enter a G_0 state, based on the cessation of DNA synthesis and a reduction in c-myc and histone H3.2 mRNA expression. The results presented here demonstrate an induction of c-fos, c-myc, and actin mRNAs by reattachment. The role of adhesion, as separate from that of growth factors, was also addressed. Adhesion in the absence of serum was shown to induce c-fos and c-myc mRNA and, thus, initiate the entry of quiescent cells into G_1. Subsequent progression of these activated cells into S phase was shown to require epidermal growth factor (EGF) and insulin and extensive spreading on an extracellular matrix. The direct involvement of components of the adhesion plaque in mechanisms that regulate growth is discussed.

METHODS

Cell Culture. BALB/c 3T3 fibroblasts, subclone A31-CL7 (A31) (gift of J. Campisi) were grown in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% calf serum and 4 mM glutamine as described (19). A31 cell stocks were discontinued after passage 13 and new cultures were started from frozen stocks.

Methocel medium (methyl cellulose 4000 centipoise, Dow Chemical) was prepared as described by Benecke *et al.* (2). Exponential-phase cells were grown to ≈70% confluence, treated with trypsin, and suspended in Methocel supplemented with 10% calf serum, 4 mM glutamine, and 10 mM Hepes at a concentration of 1.5×10^5 cells per ml. Suspended cells were harvested as described (2) and the cell pellet was resuspended in DMEM and replated onto tissue culture plastic at a concentration of 1×10^6 cells per P100 plate and 1×10^5 cells per P35 plate.

Suspension in Low Serum. A31 cells were suspended into methylcellulose containing 10% serum, harvested after 24 hr, and resuspended in methylcellulose containing 0.4% serum. After an additional 24 hr in suspension, cells were harvested and replated under a variety of different conditions. Fibronectin was obtained from the New York Blood Center (New York) and diluted with sterile phosphate-buffered saline (PBS) to a final concentration of 10 μg/ml prior to use. Plates were coated with 100 μg of fibronectin, incubated 3–6 hr at 37°C, and rinsed three times with PBS and twice with DMEM prior to replating. Insulin (Behring Diagnostics, La Jolla, CA) was used at a final concentration of 10 μg/ml and EGF (Collaborative Research, Waltham, MA) was used at a final concentration of 10 ng/ml.

Nuclear Labeling and Autoradiography. Cells were suspended as described, allowed to reattach onto fibronectin or tissue culture plastic in various media, and pulsed with 2 μCi of [^{3}H]thymidine for 24 hr (1 Ci = 37 GBq). Cells were fixed

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Abbreviation: EGF, epidermal growth factor.

with methanol and processed for autoradiography as described by Campisi *et al.* (19).

RNA Isolation and RNA Blot Analysis. Isolation and filter hybridization analysis of total cellular RNA were performed as described elsewhere (20, 21). The nitrocellulose filters were hybridized to ^{32}P-labeled cDNA clones at 0.5–1 × 10^6 cpm per lane. The various cDNA probes used were as follows: c-myc (22), c-fos (23), histone 3.2 (H3.2) (24), fibronectin (25), actin (20), and β-tubulin (RBT-3) (21). The nitrocellulose filter was autoradiographed on preflashed film.

S1 Nuclease Analysis. S1 nuclease quantitation for c-myc mRNA was performed by the method of Berk and Sharp (26) as modified by Favalaro *et al.* (27). RNA (10 μg) was hybridized for 18 hr with ^{32}P-labeled c-myc probe (30,000 cpm) and then samples were digested at 37°C with 300 units of S1 nuclease. The undigested fragments were electrophoresed on 8 M urea/6% acrylamide gel, and the gels were autoradiographed on preflashed film.

***In Vitro* Nuclear Run-On Transcription.** Nuclei were isolated and *in vitro* transcription run-on assays were carried out as described by Greenberg and Ziff (28). A31 cells were isolated after 5 min of reattachment to allow separation of cells from methylcellulose contamination. Equal numbers of counts were hybridized to nitrocellulose filters to which cDNA clones had previously attached by a slot blot technique.

RESULTS

Expression of mRNAs Corresponding to Cytomatrix and Growth-Associated Genes During Suspension of A31 Cells in Methylcellulose. The level of mRNA synthesis in Swiss 3T3 and 3T6 cells decreases to ≈20% of exponential levels within 12 hr of suspension (2); however, cells compensate for the inhibition of RNA synthesis by the stabilization of preexisting mRNA. To determine the effect of suspension on specific mRNAs in BALB/c 3T3 cells, we analyzed total RNA isolated at various times during suspension culture. mRNAs corresponding to c-myc and histone decayed extensively within 48–72 hr of suspension (Fig. 1). Expression of these genes is considered an indicator of the active growth state of the cell (15, 29). Additional studies (data not shown) revealed that DNA synthesis decreased to 2% of exponential levels within 48 hr of suspension culture as demonstrated in Swiss 3T3 cells (2).

The expression of β-tubulin and actin decreased by a factor of 4–5 in suspension culture. The levels of fibronectin mRNA, however, increased 5-fold above those measured during exponential growth. There are minor fluctuations in the abundance of some of these mRNAs at select time points, which result from discrepancies in sample loading and do not affect the analysis of overall trends in mRNA expression during the suspension period. These results indicate that during suspension culture the expression of growth-related and cytoskeletal genes was depressed; however, the cells appear to maintain the ability to selectively stimulate the expression of fibronectin genes.

Adhesion Induces the Expression of Growth-Associated Genes. To determine whether the pattern of gene expression induced in response to adhesion was similar to that seen after serum activation, the expression of c-*fos*, c-*myc*, actin, β-tubulin, and histone H3.2 genes was analyzed as biochemical markers for growth activation and progression into S phase. Adhesion of suspension-arrested A31 cells in 10% serum induced a rapid (within 30 min) and transient increase in the level of c-fos mRNA, which decreased to undetectable levels by 2 hr (Fig. 2). There was also a 10-fold increase in the relative abundance of c-myc mRNA within 2 hr of adhesion. The expression of both c-*fos* and c-*myc* followed the same apparent kinetics as those observed in serum-stimulated serum-deprived fibroblasts (28, 30).

Histone H3.2 mRNA levels accumulated during reattachment (Fig. 2) coincidental with the onset of DNA synthesis that occurred between 12 and 18 hr (data not shown) as observed in Swiss 3T3 cells (2). The level of actin mRNA was also rapidly induced in response to reattachment; undergoing two transitory peaks of induction at 1 and 4 hr and then reaching the control levels by 18–24 hr (Fig. 2). Recovery of β-tubulin mRNA expression to control levels was prolonged

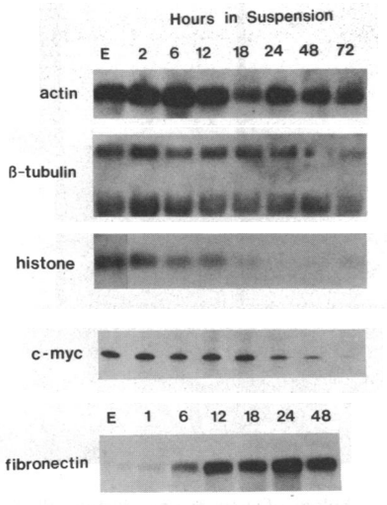

FIG. 1. Expression of specific mRNAs decreases as A31 cells enter a quiescent state in suspension. Exponential-phase A31 cells were suspended in Methocel/10% serum. Total RNA was isolated from exponential-phase cells (E) and suspended cells at 2, 6, 12, 18, 24, 48, and 72 hr by the guanidine hydrochloride method. Equal amounts of total RNA were analyzed for β-actin, fibronectin, β-tubulin, and histone H3.2 coding sequences. Steady-state levels of c-myc mRNA were quantitated by the S1 nuclease reaction.

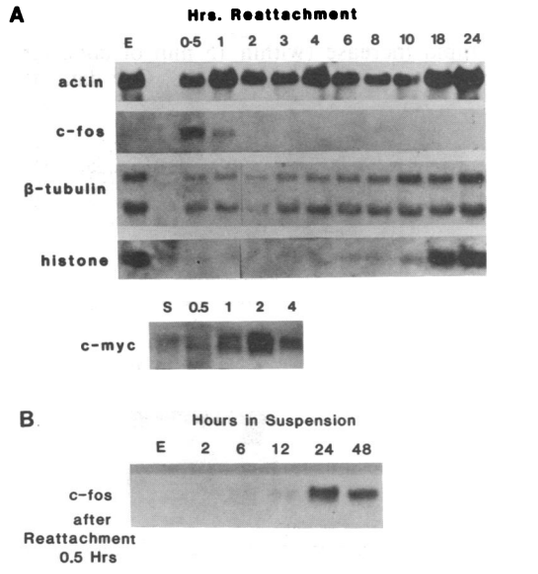

FIG. 2. Kinetics of mRNA induction during reattachment of suspended A31 cells. (*A*) Exponential-phase A31 cells (lane E) were suspended in Methocel/10% serum for 72 hr and replated in medium containing 10% serum. Total RNA was isolated at various times during reattachment (0–24 hr) and equal amounts of total RNA were analyzed for actin, c-fos, β-tubulin, histone, and c-myc by filter hybridization (RNA blot) analysis. (*B*) A31 cells were suspended in Methocel/10% serum for 2, 6, 12, 24, and 48 hr. Cells were harvested at the different times, replated for 30 min in 10% serum, and total RNA was isolated. c-fos mRNA levels were analyzed in equal amounts of RNA by filter hybridization.

throughout the reattachment period and did not reach control levels until 18–24 hr (Fig. 2). This paralleled the recovery of general mRNA synthesis as reported for suspension-arrested Swiss 3T6 cells (2, 4, 5) and coincided with the onset of DNA synthesis.

The induction of c-fos mRNA in response to adhesion was demonstrated to be directly related to the G_0/G_1 transition and not simply to the manipulations during replating (Fig. 2*B*). A31 cells were suspended for increasing periods of time and at the times shown, cells were harvested, allowed to adhere to tissue culture dishes for 30 min, and c-fos mRNA levels were measured (Fig. 2*B*). It is apparent that the extent of c-fos expression is dependent on the length of time in suspension culture, which correlates with the number of cells entering a G_0 state. A similar increase in c-fos expression in response to serum stimulation was previously shown to correlate with the length of time fibroblasts were maintained in low serum (31).

Increased Transcription of c-fos and Actin Genes in Response to Adhesion. Prolonged suspension of 3T3 fibroblasts not only arrests growth but also induces an extensive cessation of most macromolecular processes, including transcription. The induction of c-*fos* and actin gene expression in response to serum stimulation of quiescent fibroblasts was shown to be controlled exclusively at the level of transcription (28, 32, 33). We questioned, therefore, whether the expression of these genes in response to adhesion was also transcriptionally regulated, especially since previous studies by Ben-Ze'ev *et al.* (5) demonstrated that the recovery of general transcription in suspension-arrested Swiss 3T6 cells requires extensive cell spreading and coincides with S phase. Transcriptional analysis of c-*fos* and actin genes in suspension-arrested A31 cells at 5 and 15 min after reattachment was measured by *in vitro* nuclear run-on assays. Adhesion caused a rapid and specific induction of c-fos and actin transcription but had no effect on the transcriptional activity of the β-tubulin gene (Fig. 3*A*).

Fig. 3*B* further reveals that the rapid activation of transcription of c-*fos* and actin genes is accompanied by an equally rapid increase (within 15 min of adhesion) in the levels of the corresponding mRNAs. Our data, therefore, indicate that stimulation by cell substrate contact is capable of activating specific genes required for growth, even when the overall activity of the nucleus is suppressed as a result of the altered cell morphology (5).

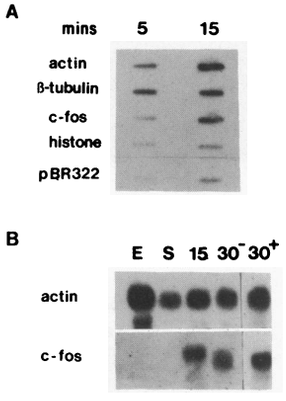

FIG. 3. Rapid induction of c-*fos* and actin gene expression by cell-surface adhesion. (*A*) A31 cells were suspended in Methocel/10% serum for 72 hr and replated in 10% serum. Nuclei were extracted by the Nonidet P-40 lysis method after 5 and 15 min of adhesion. *In vitro* transcription run-on assays were performed and the ^{32}P-labeled run-on transcripts generated were hybridized to a series of cDNA clones immobilized as slots on nitrocellulose paper. (*B*) Exponential-phase cells (lane E) were suspended in Methocel/10% serum for 72 hr, harvested, and replated in 10% serum or in 0.4% serum (lane 30⁻). Total RNA was isolated at selected times after replating and equal amounts were analyzed for c-fos mRNA, washed in 50% formamide/50% Tris EDTA to remove signal, and analyzed for actin mRNA.

Interplay Between Cell Adhesion and Growth Factors in Regulating Gene Expression. In the experiments outlined above, the only apparent variable was the extent of cell adhesion since the same concentration of serum was present during both suspension and reattachment. This fact does not necessarily mean that growth factors are not involved in the induction of gene expression. It is quite possible that the change in cell morphology may somehow alter the interaction of the cells with specific growth factors and thereby regulate growth. Fig. 3*B* (lane 30⁻) reveals that cells growth-arrested by suspension culture in 10% serum could be stimulated to induce the expression of c-fos and actin mRNAs in response to adhesion in the presence of low concentrations of serum (0.4%). During these experiments, however, it is possible that growth factors in the suspension culture medium remain associated with the cell surface and are activated during adhesion.

To define the role of adhesion as separate from that of growth factors, cells were serum deprived while in suspension culture by decreasing the serum concentration to 0.4%. Cells were then replated in the presence or absence of serum, either onto plastic or fibronectin coated plates. The overall morphology of the cells was analyzed under these conditions since we considered that these different culture environments may dramatically affect the extent of adhesion and thereby influence the ability to induce gene expression. Notably, at 30 min of adhesion there was little morphological difference at this level of analysis between all the different plating conditions (Fig. 4). As time progressed, however, the differences

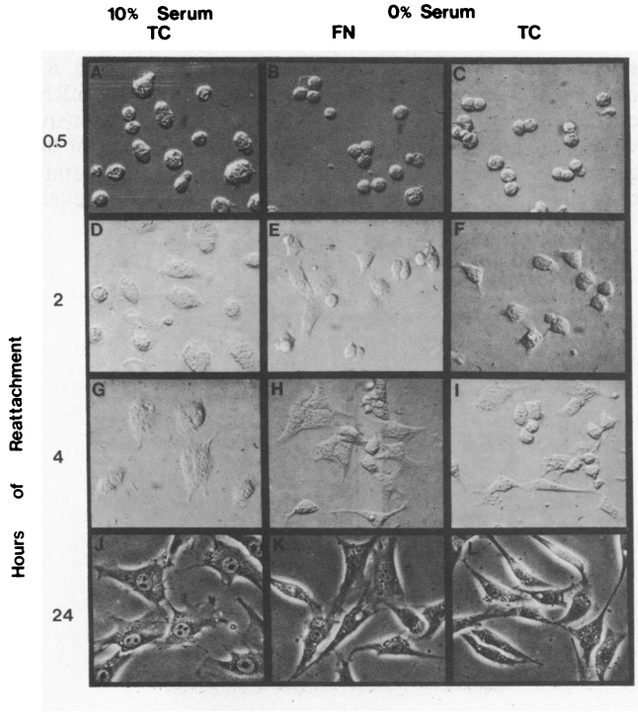

FIG. 4. Morphology of suspension-arrested cells during adhesion onto fibronectin matrix in the absence of serum. A31 cells were suspended in Methocel/0.4% serum and were replated onto tissue culture (TC) plastic in DMEM containing either 10% serum or no serum. Alternatively, cells were replated onto fibronectin (FN) matrix in the absence of serum. Cell morphology was observed after 30 min, 2, 4, and 24 hr of adhesion. Photomicrographs were taken using Hoffman optics (30 min, 2 and 4 hr) or phase-contrast optics (24 hr). (×200.)

in the rate and extent of spreading in response to serum and/or fibronectin became more apparent. After 24 hr, there was a distinct difference in the overall morphology of the cells adhering to plastic in 10% serum compared to cells on plastic in the absence of serum. Cells reattached in 10% serum spread extensively, while cells plated without serum adopted a more spindle type morphology (Fig. 4). The morphology of cells on fibronectin matrix assumed an intermediate shape. This demonstrates that there are other undefined factors in serum besides fibronectin, possibly soluble growth factors or additional extracellular matrix components, that are important for cell spreading.

Adhesion in the absence of serum onto fibronectin matrix, or when it occurred efficiently on tissue culture plastic, was able to induce the expression of the growth-related genes c-*fos* and c-*myc* (Fig. 5). As shown previously, the expression of these two genes is negligible in suspension (Figs. 2 and 3). These data suggest that the process of adhesion, independent of the presence of growth factors, is capable of influencing gene expression associated with growth activation.

Progression into DNA Synthesis Requires EGF, Insulin, and Fibronectin Matrix. The adhesion of suspension-arrested fibroblasts in the absence of serum was sufficient to activate a G_0 to G_1 transition, based on the expression of c-*fos* and c-*myc*, but was not capable of facilitating progression into S phase in the absence of additional growth factors. Cells arrested by suspension in 0.4% serum were allowed to adhere onto either tissue culture plastic or fibronectin-coated dishes in the presence of serum, EGF alone, insulin alone, or EGF and insulin together. Progression into S phase was measured by incorporation of [^{3}H]thymidine into nuclei. Addition of EGF or insulin alone was unable to induce DNA synthesis, indicating that the adhesion-activated cells exist in early G_1 (34, 35). When EGF and insulin were added together to fibroblasts plated onto tissue culture plastic, there was a small increase in the number of cells entering S phase. This number, however, increased 2-fold when cells were allowed to adhere to fibronectin in the presence of EGF and insulin (Fig. 6). It is quite apparent that EGF, insulin, and fibronectin together still only induce progression into S phase to a level 25–30% of that achieved by addition of 10% serum. In addition, these components did not facilitate the same degree of spreading as that observed in 10% serum (data not shown). These data suggest that there may be a cooperation between progression factors and adhesion onto fibronectin that is important for passage of cells through the cell cycle.

DISCUSSION

We have demonstrated that adhesion of suspension-arrested BALB/c 3T3 cells, in the presence or absence of serum, stimulated their reentry into the growth cycle with events characteristic of the G_0/G_1 transition (28, 30, 32). The suspended cells are arrested in G_0 based on the inhibition of

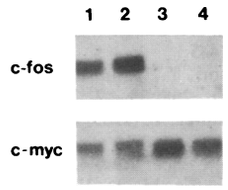

FIG. 5. Adhesion of suspension-arrested A31 cells in the absence of serum induced expression of c-*fos* and c-*myc*. A31 cells were suspended in Methocel/0.4% serum and were replated into DMEM in the absence of serum, either onto tissue culture plastic (lanes 1 and 3) or onto dishes precoated with fibronectin (10 μg/ml) (lanes 2 and 4). Total RNA isolated after 30 min (lanes 1 and 2) and 2 hr of reattachment (lanes 3 and 4) was analyzed for c-fos- and c-myc-specific sequences by filter hybridization.

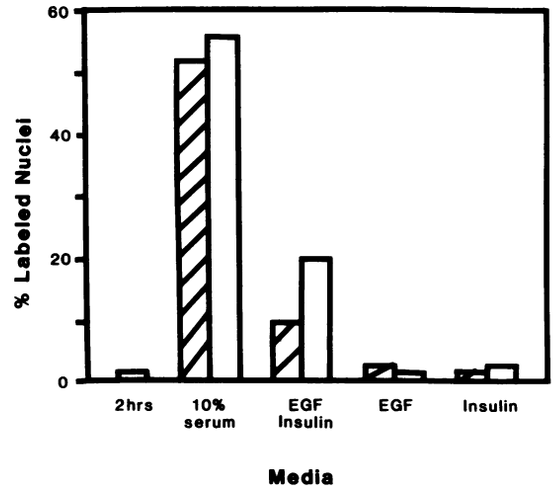

FIG. 6. Adhesion onto fibronectin in the presence of EGF and insulin is required for cells to enter DNA synthesis. Cells were suspended in Methocel/0.4% serum. After 48 hr, cells were harvested and replated in DMEM containing 10% serum, EGF (10 ng/ml) and insulin (1 μg/ml), EGF alone, or insulin alone. Cells were plated directly onto tissue culture plastic (open bars) or onto dishes precoated with fibronectin (hatched bars). Control cells were labeled for 2 hr with [^{3}H]thymidine to measure the quiescent state, while all other cells were labeled with [^{3}H]thymidine for 24 hr and processed for autoradiography.

DNA synthesis and decreased expression of c-*myc* and histone genes. This state was induced by the elimination of cell adhesion without altering other components of the culture medium such as nutrients or growth factors. The induction of the genes c-*fos* and c-*myc* occurred when cells were replated onto tissue culture plastic in the absence of additional growth factors. Furthermore, the expression of c-*fos* was enhanced when adhesion was mediated by fibronectin matrix. Adhesion of suspension-arrested cells onto fibronectin enabled an increased number of cells to respond to EGF and insulin and progress into S phase.

Fibronectin facilitates adhesion and spreading of fibroblasts on substrate and is required for cells treated with trypsin to adhere to a surface when plated in the absence of serum (36). Trypsin-treated cells that are maintained in suspension culture for more than 24 hr can adhere in serum-free conditions (Fig. 4). This may be due to the synthesis and extracellular secretion of fibronectin during suspension as indicated by the extensive increase in the level of fibronectin mRNA during suspension culture. A similar increase in fibronectin mRNA levels was not observed in cells arrested by high density (data not shown), which suggests that the expression of components involved with adhesion may result from the lack of contact with the substratum. It seems likely that fibronectin protein is produced in suspension culture and coats the cell surface. The matrix formed around the cell may then mediate adhesion during reattachment and thereby facilitate the activation of gene expression associated with G_0/G_1 transition. Fibronectin synthesis is also induced by type β transforming growth factor, a soluble growth factor that maintains growth of anchorage-dependent fibroblasts in soft agar (37). The induction of fibronectin mRNA during suspension in methylcellulose presumably involves an alternative mechanism other than type β transforming growth factor since growth is not sustained in these cells.

Previous studies addressing the morphological control of cell growth have directly correlated the extent of DNA synthesis with alterations in cell shape (8–13). Any mechanism that focuses on cell shape, however, must also consider numerous other parameters that may influence growth; for

instance, cell volume (8), membrane surface area (12), nuclear shape (38), and cytoskeletal organization (39, 40) among others. Our studies and others' (38) suggest that another important component of cell shape-dependent growth is the extent of adhesion involving the binding of ECM proteins to transmembrane receptors.

Studies have shown that there are several second messenger pathways by which soluble growth factors can stimulate growth and gene expression (41, 42). These pathways may involve a series of phosphorylation events that are dependent on the activation of protein kinases (14, 15, 43). It is possible that adhesion may induce gene expression associated with the G_0 to G_1 transition through one or more of these systems.

Alternatively, the cytoskeleton, whose organization may be altered during the process of adhesion, may be involved in signal transduction to the nucleus (40, 44). Several studies have indicated that microtubules and microfilaments may act directly to regulate growth (40), and a recent study (18) demonstrated that activation of density-arrested cells with platelet-derived growth factor resulted in the rapid dissociation of vinculin and actin stress fibers from the adhesion plaque, the site of attachment to the extracellular matrix in spread fibroblasts. Evidence (45) suggests that particular components of the adhesion plaque may be targets for second messenger enzyme activity, such as those requiring a Ca^{2+} influx. Although the role of the cytoskeleton in growth activation is unclear, it has important implications for fibronectin-induced gene expression. Microfilaments are associated with the extracellular matrix components at the adhesion plaque through the transmembrane protein integrin (43, 46). Phosphorylation events occurring at the adhesion plaque (43, 47) may contribute to the transduction of mitogenic signals to the nucleus by altering the organization of the cytoskeleton. This mechanism could involve a direct cytoskeletal pathway to the nucleus or it may affect the availability of soluble growth factor receptors for their ligands.

Our study demonstrates that fibronectin facilitates the progression of cells in the growth cycle. While it has been suggested that fibronectin and other ECM components may directly affect gene expression, to our knowledge this has never been directly demonstrated. Growth stimulation and gene activation by the ECM cannot be adequately addressed in serum-deprived and confluent fibroblasts because the ECM is an abundant and constant component in these tissue culture systems. Receptors for matrix components in these cells would be unavailable for new stimulation except potentially during the process of wounding a fibroblast monolayer. In fact, studies have demonstrated that in the absence of serum factors, wounding stimulates c-*fos* expression in those cells peripheral to the wound (48). The altered adhesion of cells in the wound could possibly direct the stimulus for gene expression.

We suggest that the suspension system outlined in these studies provides a unique means of analyzing the role of the ECM, its receptors, and its interaction with the microfilament system in the activation of gene expression during growth.

We thank Dr. Judith Campisi for providing the BALB/c-3T3 (clone A31-CL7) cell line and for her valuable advice during the initial phases of this work. We also thank Dr. Richard Hynes for the fibronectin cDNA clone. We are grateful to Drs. Avri Ben-Ze'ev, Gail Sonenshein, and Paula Traktman for critical reading of the manuscript and useful suggestions. This work was supported by National Institutes of Health Grant GM29630.

1. Stoker, M., O'Neill, C., Berryman, S. & Waxman, V. (1968) *Int. J. Cancer* **3**, 683–693.
2. Benecke, B. J., Ben-Ze'ev, A. & Penman, S. (1978) *Cell* **14**, 931–939.
3. Farmer, S. R., Ben-Ze'ev, A., Benecke, B. J. & Penman, S. (1978) *Cell* **15**, 627–637.
4. Benecke, B. J., Ben-Ze'ev, A. & Penman, S. (1980) *J. Cell. Physiol.* **103**, 247–254.
5. Ben-Ze'ev, A., Farmer, S. R. & Penman, S. (1980) *Cell* **21**, 365–372.
6. Wittelsberger, S. C., Kleene, K. & Penman, S. (1981) *Cell* **24**, 859–866.
7. Tucker, R. S., Butterfield, C. E. & Folkman, J. (1981) *J. Supramol. Struct. Cell. Biochem.* **15**, 29–40.
8. Folkman, J. & Greenspan, H. P. (1975) *Biochim. Biophys. Acta* **417**, 211–236.
9. Otsuka, H. & Moskowitz, M. (1975) *J. Cell. Physiol.* **87**, 213–220.
10. Folkman, J. & Moscona, A. (1978) *Nature (London)* **273**, 345–349.
11. Gospodarowicz, D., Greenburg, G. & Birdwell, C. R. (1978) *Cancer Res.* **38**, 4155–4171.
12. O'Neill, C. H., Riddle, P. N. & Jordan, P. W. (1979) *Cell* **16**, 909–918.
13. O'Neill, C., Jordan, P. & Ireland, G. (1986) *Cell* **44**, 489–496.
14. Nishizuka, V. (1984) *Nature (London)* **308**, 693–698.
15. Rozengurt, E. (1986) *Science* **234**, 161–166.
16. Bissell, M. J., Hall, G. H. & Parry, G. (1982) *J. Theor. Biol.* **99**, 31–68.
17. Kleinman, H. K., Klebe, R. J. & Martin, G. R. (1981) *J. Cell Biol.* **88**, 473–485.
18. Herman, B. & Pledger, W. J. (1985) *J. Cell Biol.* **100**, 1031–1040.
19. Campisi, J., Morreo, G. & Pardee, A. B. (1984) *Exp. Cell Res.* **152**, 459–466.
20. Bond, J. F. & Farmer, S. R. (1983) *Mol. Cell. Biol.* **3**, 1333–1342.
21. Bond, J. F., Robinson, G. S. & Farmer, S. R. (1984) *Mol. Cell. Biol.* **4**, 1313–1319.
22. Dean, M., Kent, R. B. & Sonenshein, G. (1983) *Nature (London)* **305**, 443–446.
23. Curran, T., Peters, G., van Beveren, C., Teich, N. M. & Verma, I. (1982) *J. Virol.* **44**, 674–682.
24. DeLisle, A. J., Graves, R. A., Marzluff, W. F. & Johnson, L. F. (1983) *Mol. Cell. Biol.* **3**, 1920–1929.
25. Schwarzbauer, J. E., Tamkun, J. W., Lemischka, I. R. & Hynes, R. O. (1983) *Cell* **35**, 421–431.
26. Berk, A. J. & Sharp, P. A. (1977) *Cell* **12**, 721–732.
27. Favalaro, J., Triesman, R. & Kamen, R. (1980) *Methods Enzymol.* **65**, 718–749.
28. Greenberg, M. E. & Ziff, E. B. (1984) *Nature (London)* **311**, 433–437.
29. Schumperti, D. (1986) *Cell* **45**, 471–472.
30. Muller, R., Bravo, R., Burckhardt, J. & Curran, T. (1984) *Nature (London)* **312**, 716–720.
31. Bravo, R., Burchhardt, J., Curran, T. & Muller, R. (1986) *EMBO J.* **5**, 695–700.
32. Greenberg, M. E., Hermanowski, A. L. & Ziff, E. B. (1986) *Mol. Cell. Biol.* **6**, 1050–1057.
33. Elder, P. K., Schmidt, L. O., Ono, T. & Getz, M. J. (1984) *Proc. Natl. Acad. Sci. USA* **81**, 7476–7480.
34. Scher, C. D., Shepard, R. C., Antoniades, H. N. & Stiles, C. D. (1979) *Biochem. Biophys. Acta* **560**, 217–241.
35. Pardee, A. B., Dubrow, R., Hamlin, J. L. & Kletzien, R. F. (1978) *Annu. Rev. Biochem.* **47**, 715–750.
36. Akiyama, S. K. & Yamada, K. M. (1986) *Adv. Enzymology* **59**, 2–57.
37. Ignotz, R. A. & Massague, J. (1986) *J. Biol. Chem.* **261**, 4337–4345.
38. Ingber, D. E., Madri, J. A. & Folkman, J. (1987) *In Vitro Cell. Dev. Biol.* **23**, 387–394.
39. Farmer, S. R., Wan, K. M., Ben-Ze'ev, A. & Penman, S. (1983) *Mol. Cell. Biol.* **3**, 182–189.
40. Farmer, S. R. (1986) in *Cell and Molecular Biology of the Cytoskeleton*, ed. Shay, J. W. (Plenum, New York), pp. 131–149.
41. McCaffrey, P., Ran, W., Campisi, J. & Rosner, M. R. (1987) *J. Biol. Chem.* **262**, 1442–1445.
42. Tsuda, T., Hamamori, Y., Yamashita, T., Fukomoto, Y. & Takai, Y. (1986) *FEBS Lett.* **208**, 39–42.
43. Cooper, J. A. & Hunter, T. (1983) *Curr. Top. Microbiol. Immunol.* **107**, 125–162.
44. Burridge, K. (1986) *Cancer Rev.* **4**, 18–78.
45. Herman, B., Harrington, M. A., Vlashaw, N. E. & Pledger, W. J. (1986) *J. Cell. Physiol.* **126**, 115–125.
46. Hynes, R. O. (1987) *Cell* **48**, 549–554.
47. Hirst, R., Horwitz, A., Buck, C. & Rohrschneider, L. (1986) *Proc. Natl. Acad. Sci. USA* **83**, 6470–6474.
48. Verrier, B., Muller, D., Bravo, R. & Muller, R. (1986) *EMBO J.* **5**, 913–917.