Integrin Phosphorylation Is Modulated during the Differentiation of F-9 Teratocarcinoma Stem Cells

Stephen C. Dahl and Laura B. Grabel
Department of Biology, Wesleyan University, Middletown, Connecticut 06457

Abstract. The retinoic acid-induced differentiation of F-9 teratocarcinoma cells in monolayer culture is accompanied by the accumulation of fibrillar fibronectin deposits, the appearance of a highly structured actin cytoskeleton, and the redistribution of integrin to apparent sites of substrate contact. We have studied the 140-kD fibronectin receptor during this process and report that although the integrin molecule is present in equivalent amounts before and after differentiation, the level of integrin phosphorylation decreases dramatically as the cells differentiate. This loss of phosphorylation coincides temporally with the observed changes in actin, fibronectin, and integrin organization. The phosphorylation state of integrin thus may mediate developmentally regulated cell-matrix interactions.

The regulation of fibronectin matrix deposition and the cellular response to these extracellular matrices are important for a number of developmental events, including cell adhesion, migration, and differentiation (Chiuet et al., 1979; Rovasio et al., 1983; Grabel and Watts, 1987; Patel and Lodish, 1987). Because the cellular response to fibronectin often involves alterations in cell shape, it has been suggested that the cytoskeleton may be involved in the coordination of these interactions (Hynes, 1981; Burridge, 1986). This hypothesis is supported, in part, by immunofluorescence and EM studies that demonstrate the colocalization of extracellular fibronectin fibers and the actin-based cytoskeleton (Hynes and Destree, 1978; Singer, 1979). These data predict the existence of a transmembrane receptor link between the extracellular matrix and the cytoskeleton.

One class of receptors that appears to provide such a transmembrane linkage is the family of fibronectin receptors known as integrins (for recent reviews see Buck and Horwitz, 1987; Hynes, 1987). Integrins are moderate-affinity fibronectin receptors that exist as oligomeric complexes composed of three subunits in avian systems and two subunits in mammalian systems (Hynes, 1987). Like fibronectin, integrins have also been shown to colocalize with cytoskeletal actin, particularly in areas of focal contacts (Chen et al., 1985; Damsky et al., 1985). This interaction may be mediated by the integrin complexes' ability to bind to talin, a high molecular weight component of focal contacts that appears to participate in the docking of actin filaments terminating at the plasma membrane (Burridge and Connell, 1983; Horwitz et al., 1986). The association of integrin with cytoskeletal and extracellular matrix components makes this family of fibronectin receptors a likely interface for coordinating cell-matrix interactions.

A clue to the mechanism whereby integrin might modulate intracellular events may be found in the amino acid sequence of its subunits. It has been shown that the presumed cytoplasmic domain of the integrin beta chain shares considerable homology with the cytoplasmic domains of both the epidermal growth factor and insulin receptors including the consensus tyrosine phosphorylation site (Tamkun et al., 1986). The regulation of integrin phosphorylation at this site may be a mechanism for controlling fibronectin receptor function by altering integrin-cytoskeleton or integrin-fibronectin interactions (Tamkun et al., 1986; Buck and Horwitz, 1987). Recently, it has been shown that the transformation of avian fibroblasts with Rous sarcoma virus (RSV) results in the phosphorylation of integrin bands 2 and 3 (Hirst et al., 1986). The RSV-transformed phenotype is also characterized by the loss of cell-associated fibronectin, disorganization of the actin-based cytoskeleton, and altered cell adhesive properties (Burridge, 1986). These phenotypic transitions may be the result of integrin phosphorylation (Hirst et al., 1986; Tamkun et al., 1986). This hypothesis has been further supported by data demonstrating that phosphorylated integrin has a lower binding affinity for fibronectin and talin as assayed by equilibrium gel filtration experiments (Buck and Horwitz, 1987). It remains to be seen, however, if the changes in integrin phosphorylation observed for RSV-transformed cells are also a mechanism for altering cell-substrate adhesion and fibronectin matrix formation in normal cells during development.

S. C. Dahl's present address is Department of Cellular and Developmental Biology, Harvard University, Cambridge, MA 02138.

1. Abbreviation used in this paper: RSV, Rous sarcoma virus.
When monolayers of F-9 teratocarcinoma stem cells are cultured in the presence of retinoic acid, they differentiate into parietal endoderm and acquire the ability to accumulate fibrous deposits of fibronectin (Strickland and Mahdavi, 1978; Linder et al., 1981). This change in fibronectin deposition does not appear to be the result of alterations in fibronectin structure or the levels of its synthesis (Dahl and Grabel, 1988). We now demonstrate that the ability of differentiating F-9 cells to assemble these fibronectin fibers is temporally correlated with the loss of integrin phosphorylation, the appearance of a highly structured actin cytoskeleton, and the redistribution of integrin to sites of apparent substrate contact. This developmentally controlled modulation of integrin phosphorylation may mediate structural changes associated with parietal endoderm differentiation.

Materials and Methods

Cell Culture

Initial cultures of F-9 teratocarcinoma stem cells and all-trans retinoic acid were the generous gift of Dr. S. Strickland. Cells were cultured as described previously (Dahl and Grabel, 1988). Briefly, monolayer cultures were induced to differentiate into parietal endoderm by the addition of 0.1 µM retinoic acid solubilized in absolute ethanol. Cells were treated for three consecutive days to ensure a differentiating population, and all manipulations were performed in subdued light.

Immunofluorescence

Cells were plated on 18 × 18 mm glass coverslips and cultured with or without retinoic acid treatment. To colocalize actin and fibronectin, cell layers were washed three times with PBS, and fixed for 10 min with 3.7% formalin in PBS followed by 5 minutes in acetone at −20°C. Coverslips were then air dried and incubated for 30 min with a 1:10 dilution of affinity-purified anti-gpl40 (integrin) serum (Brown and Juliano, 1986; generous gift of Dr. P. Brown, University of Texas Medical School, Houston, TX). The nitrocellulose sheets were then washed twice with Blotto (1:500 dilution of goat anti-140-kD receptor antibody in Blotto, Brown and Juliano, 1986; generous gift of Dr. P. Brown, University of Texas Medical School, Houston, TX). The first two washes of Pansorbin-bound material were also performed in NET buffer. All integrin and talin samples were then solubilized with 50 µl sample buffer (80 mM Tris-HCl pH 6.8, 2% SDS, 5% 2-mercaptoethanol) for 5 min, and electrophoresed on 7.5% SDS-polyacrylamide gels (Laemmli, 1970). Gels were dried down and exposed to XAR-5 film (Eastman Kodak Co., Rochester, NY) in the presence of an enhancing screen (Cronex; DuPont, Wilmington, DE). For fluorography of [35S]-labeled material, gels were treated with Enlightening (New England Nuclear) before being dried and exposed to x ray film.

Immunoblots

Lysates of nonlabeled F-9 cells were prepared as described above and the material from equal cell numbers was processed for SDS-PAGE on 7.5% gels. The proteins were then electrophoresed onto nitrocellulose paper (Schleicher & Schuell, Keene, NH) by the method of Towbin et al. (1979). The nitrocellulose sheets were blocked with 5% nonfat dry milk (Biostat) and incubated overnight with a 1:500 dilution of goat anti-140-kD receptor antibody in Blotto (Brown and Juliano, 1986; generous gift of Dr. P. Brown, University of Texas Medical School, Houston, TX). The nitrocellulose sheets were then washed twice with Blotto for 15 min each and incubated for 2 h with a 1:10,000 dilution of peroxidase conjugated rabbit anti-goat antibody in Blotto (Cappel Laboratories). After this incubation, the nitrocellulose sheets were washed twice with Tris-buffered saline (50 mM Tris-HCl, 200 mM NaCl, pH 7.4) for 15 min each, and the localized proteins visualized with 0.5 mg/ml 4-chloro-l-napthol and 0.003% hydrogen peroxide in Tris-buffered saline.

Phosphoamino Acid Analysis

Phosphoamino acid analysis was performed as described by Kellie et al. (1986) with minor modifications. Briefly, 32P-labeled fibronectin receptor was cut from dried gels of immunoprecipitated material using the autoradiograph as a template. The excised gel slices were rehydrated in 50 mM ammonium bicarbonate containing 0.1% SDS and 5% 2-mercaptoethanol, boiled for 5 min, and incubated with shaking for 16 h at 37°C. The solubilized proteins were then TCA precipitated with 25 µg BSA added as a carrier, washed with cold ethanol, lyophilized, and hydrolyzed in 6 N HCl (Pierce Chemical Co., Rockford, IL) for 2 h. The samples were then lyophilized, washed twice with distilled water, and loaded onto cellulose TLC plates (EM Science, Cherry Hill, NJ). One-dimensional thin-layer electrophoresis was performed as described, but at 300 V for 1 h. Autoradiography was performed as described above and the TLC plates sprayed with ninhydrin to visualize phosphoamino acid standards (Sigma Chemical Co.).

Results

The Colocalization of Actin and Fibronectin, and Localization of Integrin

It has previously been reported that the formation of actin stress fibers accompanies the differentiation of F-9 teratocarcinoma cells into parietal endoderm upon induction with retinoic acid (Lehtonen et al., 1983). Because fibrillar fibronectin deposits have also been shown to accompany this differentiation process (Linder et al., 1988; Dahl and Grabel, 1988), we investigated the temporal and spatial appearance of these organized structures with double-label fluorescence.
microscopy. Nontreated and treated cells were stained for actin with rhodamine phalloidin and fibronectin with fluorescein-conjugated goat antihuman fibronectin after 7 d in culture (Fig. 1). The localization of actin in nontreated cells reveals short actin filaments with the greatest fluorescence intensity concentrated at the cell periphery (A). The accumulation of fibronectin in these cells is punctate and mostly membrane associated (A'). Treated cells, however, display a highly structured array of thick actin filaments throughout the cytoplasm (B) and accumulate fibrillar deposits of fibronectin (B'). As has been reported in other systems, actin and fibronectin structures colocalize in F-9 cells.

To monitor the distribution of integrin during differentiation, indirect immunofluorescence was performed using an antibody directed against the integrin beta subunit (Brown and Juliano, 1986). These data show that integrin is present in a diffuse pattern throughout the cytoplasm of day 7 stem cell cultures with apparent concentrations at regions of cell-cell contact (Fig. 1 C). Treated cells, however, display discrete areas of staining localized to apparent sites of substrate contact (D) at day 7. A reorganization of integrin distribution thus occurs during F-9 differentiation, and this reorganization also reflects the changes observed for actin and fibronectin.

As we have previously reported that fibrillar deposits of fibronectin appear between days 3 and 5 after retinoic acid treatment, we studied the temporal appearance of organized actin structures and the distribution of integrin during the differentiation of treated cultures. Fig. 2 shows rhodamine phalloidin staining for actin in treated cells after (A) 1, (B) 2, (C) 3, and (D) 4 days of culture. A similar localization of integrin is shown in Fig. 2, E, F, G, and H. Although the analysis of these data is of a qualitative nature, it appears that a highly structured actin cytoskeleton begins to be assembled

Figure 1. The localization of actin, fibronectin, and integrin on nontreated and treated F-9 teratocarcinoma cells. Nontreated cells (A, A', and C) and treated cells (B, B', and D) were grown on coverslips, fixed, and stained for fluorescence microscopy with either rhodamine-conjugated phalloidin (A and B) and fluorescein-conjugated goat antihuman fibronectin antiserum (A and B'), or IgG-purified goat antiantiintegrin serum followed by affinity-purified rhodamine-conjugated rabbit antigoat serum (C and D) after 7 d of culture. Bars, 10 μm.
3 days after retinoic acid treatment, which agrees with the data reported by Lehtonen et al. (1983). Integrin distribution also begins to be reorganized at this time, with the appearance of many presumed sites of substrate contact within 4 days of retinoic acid treatment.

**Integrin and Its Phosphorylation**

Because the integrin complex is a probable link between extracellular fibronectin and intracellular actin, we further investigated the presence of this receptor in F-9 cells before and after retinoic acid treatment. Fig. 3 depicts immunoblots...
and immunoprecipitations that were performed on cell lysates from equal numbers of non-treated and treated cells after 4 d of culture. Lanes A and B are immunoblots of material from equal numbers of (A) nontreated and (B) treated cells. Lanes C, D, E, and F are immunoprecipitates of nontreated (C and D) and treated (E and F) cell lysates incubated with immune (C and E) and preimmune (D and F) antiserum. One protein band is resolved on these blots and gels which represents the integrin pattern of integrin mobility; two bands corresponding to the alpha and beta chains of the receptor complex. These data suggest that the two cell types are synthesizing equivalent amounts of receptor at the time that fibronectin fibers begin to appear in treated cells. The presence or absence of the integrin complex thus does not account for the difference in fibronectin or actin organization observed between the two cell types.

Although integrin is present in both nontreated and treated cells, it is possible that the receptor's function could be modulated, perhaps through phosphorylation. To test this hypothesis, F-9 cell cultures were labeled with $^{35}$S]methionine and immunoprecipitations of integrin from cell extracts of nontreated and treated F-9 cells. Data shown are blots or fluorographs of one-dimensional 7.5% SDS–polyacrylamide gels. Lanes A and B represent immunoblots of material from equal numbers of (A) nontreated and (B) treated cells after 4 d of culture. Lanes C–H represent material immunoprecipitated with immune antiserum (C, E, G, and H) or preimmune antiserum (D and F) from lysates of untreated (C, D, and G) or treated (E, F, and H) cells metabolically labeled with $^{35}$S]methionine after 4 d of culture. All of the lanes contain material isolated from equal cell numbers.

Figure 3. Immunoblot and immunoprecipitations of integrin from cell extracts of nontreated and treated F-9 cells. Data shown are blots or fluorographs of one-dimensional 7.5% SDS–polyacrylamide gels. Lanes A and B represent immunoblots of material from equal numbers of (A) nontreated and (B) treated cells after 4 d of culture. Lanes C–H represent material immunoprecipitated with immune antiserum (C, E, G, and H) or preimmune antiserum (D and F) from lysates of untreated (C, D, and G) or treated (E, F, and H) cells metabolically labeled with $^{35}$S]methionine after 4 d of culture. All of the lanes contain material isolated from equal cell numbers.

To determine when the change in integrin phosphorylation occurs during differentiation, cell cultures were labeled with $^{32}$P]orthophosphate 1, 2, 3, and 4 d after treatment with retinoic acid. Fig. 5 shows immunoprecipitations of cell extracts from this series of cultures. Each pair (A and B) represents reduced material immunoprecipitated from equal numbers of (A) nontreated and (B) treated cells. A comparison of A and B for each day after plating suggests that the level of integrin phosphorylation is the same for both cell types until sometime between days 3 and 4, when it drops off dramatically in the differentiating cultures. This loss of integrin phosphorylation coincides temporally with the redistribution of integrin and the changes observed in actin structure.

To determine which amino acid residue is phosphorylated in stem cell cultures, the alpha and beta integrin bands were excised from one-dimensional nonreduced SDS-PAGE gels of immunoprecipitated $^{32}$P-labeled material and subjected to phosphoamino acid analysis. Fig. 6 shows the results of this experiment and demonstrates that the integrin phosphorylation is on serine residues. Only trace amounts of threonine and tyrosine phosphorylation were detected.

Discussion

A central problem in cell and developmental biology is how environmental changes such as interactions with the extracellular matrix can alter cell phenotype and the pattern of gene expression. It has been suggested that changes in cell shape mediated by cytoskeletal alterations may be involved in these transitions (Ben-Ze'ev et al., 1980; Benya et al., 1988; Casanova and Grabel, 1988). The mechanism whereby extracellular matrix and cytoskeleton interact, however, has yet to be elucidated. Because of its location at the interface between these two structures, the integrin molecule is a likely candidate for transmitting information between the intracellular and extracellular environment.

We have used the F-9 teratocarcinoma system to study the process of cell-matrix interaction during the differentiation of F-9 stem cells into parietal endoderm. In the developing mouse embryo, parietal endoderm is derived from the inner cell mass. Thus, it is interesting to note that the differentiation of inner cell mass cells into another cell type, trophoderm, is also accompanied by a change in integrin distribu-
tion from a cell surface concentrated pattern to localized sites of substrate adhesion (Sutherland et al., 1988).

The data presented here demonstrate that the appearance of a structured actin cytoskeleton, the redistribution of integrin to substrate contact sites, and the loss of integrin phosphorylation are all associated with the differentiation of F-9 cells to parietal endoderm. Because this also corresponds to the time that we begin to identify fibronectin fibers by indirect immunofluorescence (Dahl and Grabel, 1988), these alterations in cell structure and phosphorylation state may initiate and/or regulate the accumulation of fibrillar fibronectin deposits during parietal endoderm differentiation.

It has previously been demonstrated that avian fibroblasts transformed with RSV lose cell surface fibronectin and exhibit disorganized actin microfilaments (Edelman and Yabara, 1976; Weckus et al., 1974; Hynes and Wyke, 1975). Recently, it has been shown that RSV transformation also results in the phosphorylation of the avian integrin complex on serine and tyrosine residues (Hirst et al., 1986). Since pp60src is a known tyrosine kinase that localizes to focal contacts (Rohrschneider, 1980), the changes observed in RSV-transformed cells have been suggested to be attributable to integrin tyrosine phosphorylation (Hirst et al., 1986; Tamkun et al., 1986; Buck and Horwitz, 1987). Further support for this hypothesis exists in the published sequence of the integrin band 3 (beta chain), which reveals a tyrosine phosphorylation consensus sequence analogous to that of the epidermal growth factor receptor on the cytoplasmic region of the molecule (Tamkun et al., 1986). Our data, however, suggest the intriguing possibility that serine phosphorylation, not tyrosine, may mediate the observed effect. Toward this end, it is important to note that a serine residue is located only two amino acids away from the tyrosine described above (Tamkun et al., 1986).

The data presented here are the first demonstration of a developmentally linked phosphorylation change of the integrin complex. This change in phosphorylation may be involved in triggering the differentiation process or may merely reflect

Figure 4. Integrin and talin phosphorylation. Lanes A and B are total [32P]orthophosphate-labeled material from equal numbers of (A) nontreated and (B) treated cells after 4 d of culture. Lanes D, E, and G represent material immunoprecipitated from [32P]orthophosphate labeled lysates of (D and G) nontreated and (E) treated cells after 4 d of culture. Lanes D and E represent reduced material isolated from equal cell numbers. Lane G represents nonreduced material. Lanes C and F are immunoprecipitations of [35S]methionine-labeled cell lysates that were run as markers for (C) reduced and (F) nonreduced integrin. Lanes H-K represent material immunoprecipitated for talin from equal numbers of (H and I) [35S]methionine and (J and K) 32P-labeled (H and J) nontreated and (J and K) treated F-9 cells.
References

Ben-Ze'ev, A., S. R. Farmer, and S. Pennman. 1980. Protein synthesis requires cell-surface contact while nuclear events respond to cell shape in anchorage-dependent fibroblasts. Cell. 21:365-372.

Benya, P. D., P. D. Brown, and S. R. Padilla. 1988. Microfilament modification of dimethylcysteatin B causes retinoic acid-modulated chondrocytes to reexpress the differentiated collagen phenotype without a change in shape. J. Cell Biol. 106:161-170.

Brown, P. J., and R. L. Juliano. 1986. Expression and function of a putative cell surface receptor for fibronectin in hamster and human cell lines. J. Cell Biol. 103:1595-1603.

Buck, C. A., and A. F. Horwitz. 1987. Cell surface receptors for extracellular matrix molecules. Annu. Rev. Cell Biol. 3:179-205.

Burrage, K. 1986. Substrate adhesions in normal and transformed fibroblasts: organization and regulation of cytoskeletal, membrane and extracellular matrix components at focal contacts. Cancer Rev. 4:18-78.

Burrage, K., and L. Connell. 1983. Talin: a cytoskeletal component concentrated in adhesion plaques and other sites of actin membrane interaction. Cell Motil. 3:405-417.

Burrage, K., and P. Mangeat. 1984. An interaction between vinculin and talin. Nature (London). 308:744-746.

Casanova, J. E., and L. B. Grabel. 1988. The role of cell interactions in the differentiation of teratocarcinoma-derived parietal and visceral endoderm. Dev. Biol. In press.

Chen, W. T., T. Hasegawa, C. Hasegawa, C. Weinstock, and K. M. Yamada. 1985. Development of cell surface linkage complexes in cultured fibroblasts. J. Cell Biol. 109:1103-1114.

Chiquet, M., E. C. Puri, and D. C. Turner. 1979. Fibronectin mediates attachment of chicken myoblasts to a gelatin-coated substratum. J. Biol. Chem. 254:5475-5482.

Collett, M. S., S. K. Belzer, and A. F. Purchio. 1984. Structurally and functionally modified forms of pp60-src in Rous Sarcoma virus-transformed cell lysates. Mol. Cell. Biol. 4:1213-1220.

Dahl, S. C., and L. B. Grabel. 1988. Altered accumulations of fibronectin are not dependent on fibronectin modifications during the differentiation of F-9 teratocarcinoma stem cells. Exp. Cell Res. 176:234-247.

Damsky, C. M., K. A. Knudsen, D. Bradley, C. A. Buck, and A. F. Horwitz. 1985. Distribution of the cell-substratum attachment (CSAT) antigen on myogenic and fibroblastic cells in culture. J. Cell Biol. 100:1528-1539.

Edelman, G. M., and I. Yahara. 1976. Temperature-sensitive changes in surface modulating assemblages of fibroblasts transformed by mutants of Rous Sarcoma virus. Proc. Natl. Acad. Sci. USA. 73:2047-2051.

Grabel, L. B., and T. D. Watts. 1987. The role of extracellular matrix in the migration and differentiation of parietal endoderm from teratocarcinoma embryoid bodies. J. Cell Biol. 105:441-448.

Hirt, R., A. Horwitz, C. Buck, and L. Rohrschneider. 1986. Phosphorylation of the fibronectin receptor complex in cells transformed by oncoproteins that encode tyrosine kinases. Proc. Natl. Acad. Sci. USA. 83:6470-6474.

Hogan, B. L. M., A. Taylor, and E. D. Adamson. 1981. Cell interactions modulate embryonal carcinoma cell differentiation into parietal or visceral endoderm. Nature (London). 291:235-237.

Horwitz, A., K. Duggan, C. Buck, M. C. Beckerle, and K. Burrage. 1986. Interaction of plasma membrane fibronectin receptor with talin—a transmembrane linkage. Nature (London). 320:531-533.

Hynes, R. O. 1981. Relationships between fibronectin and the cytoskeleton. Cell Surface Rev. 7:100-137.

Hyne, R. O. 1987. Integrins: a family of cell surface receptors. Cell. 48:549-554.

Hyne, R. O., and A. T. Destree. 1978. Relationships between fibronectin (LETS Protein) and actin. Cell. 15:875-886.

Hyne, R. O., and A. J. Wyke. 1975. Alterations in surface proteins in chicken cells transformed by temperature-sensitive mutants of Rous Sarcoma virus. Virology. 64:492-504.

Johnson, D. A., J. W. Gauthus, J. R. Sportsman, and J. H. Elder. 1984. Improved technique utilizing nonfat dry milk for analysis of proteins and nucleic acids transferred to nitrocellulose. Gene Anal. Tech. 1:3-8.

Kelle, S. B. Patel, A. Mitchell, D. R. Critchley, N. M. Wigglesworth, and A. F. Purchio. 1984. Comparison of the relative importance of tyrosine-specific vinculin phosphorylation and the loss of surface-associated fibronectin in the morphology of cells transformed by Rous Sarcoma virus. J. Cell Biol. 82:129-142.

Knudsen, K. A., P. E. Rao, C. H. Damsky, and C. A. Buck. 1981. Membrane glycoproteins involved in cell-substratum adhesion. Proc. Natl. Acad. Sci. USA. 78:4071-4075.

Laemmli, U. K. 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature (London). 227:680-685.

Lehtonen, E., V. P. Lehto, R. A. Badley, and I. Virtanen. 1983. Formation of vinculin plaques precedes other cytoskeletal changes during retinoic acid-induced teratocarcinoma cell differentiation. Exp. Cell Res. 144:191-197.

Linder, U., R. Kron Dahl, R. Sennerstam, and N. R. Ringertz. 1981. Retinoic acid-induced differentiation of F9 embryonal carcinoma cells. Exp. Cell Res. 132:453-460.

Panel, V. P., and H. F. Lodish. 1986. The fibronectin receptor on mammalian erythroid precursor cells: characterization and developmental regulation. J.

Figure 6. Phosphoamino acid analysis of integrin immunoprecipitated from nontreated F-9 cells. Immunoprecipitations of [32P]orthophosphate-labeled cell lysates were processed for amino acid analysis and separated by thin-layer electrophoresis. Phosphoamino acid standards were visualized with ninhydrin and their positions marked as shown.

The transition of a stem cell to an endoderm cell capable of forming extensive contacts with a substrate in any event, the temporal correlation of integrin phosphorylation state with the redistribution of actin, fibronectin, and integrin molecules suggests that integrin phosphorylation may play a role in the organization of these proteins. Although the actual mechanism whereby phosphorylation may affect cytoskeletal and extracellular matrix organization remains to be determined, evidence that integrin phosphorylation may alter the function of the molecule exists in the data of Buck and Horwitz (1987), who have demonstrated that phosphorylated integrin has a lower affinity for talin as well as fibronectin. As integrin phosphorylation appears to be developmentally regulated in F-9 teratocarcinoma cells, this system promises to be useful in determining its role in morphogenesis.

Figure 6

We thank Dr. Patricia Brown and Dr. Vikram Patel for their generous gifts of antimammalian integrin serum; Dr. Keith Burrage for his generous gift of antimammalian talin serum; Joe Stabnick, Karin Hunsicker, and Sandy Becker for technical assistance; and Michael Tibbets and Mark Donovan for their assistance with phosphoamino-acid analysis.

This work was supported by ACS grant CD-314. S. C. Dahl is the recipient of a Connecticut High Technology Fellowship. L. B. Grabel is the recipient of a National Institutes of Health Research Career Development Award CA-01065.

Received for publication 10 May 1988, and in revised form 25 August 1988.

Dahl and Grabel Integrin Phosphorylation in F-9 Cells

189
Patel, V. P., and H. F. Lodish. 1987. A fibronectin matrix is required for differentiation of murine erythroleukemia cells into reticulocytes. J. Cell Biol. 105:3105–3118.

Rohrschneider, L. 1980. Adhesion plaques of Rous Sarcoma virus-transformed cells contain the src gene product. Proc. Natl. Acad. Sci. USA. 77:3514–3518.

Rohrschneider, L., and M. J. Rosok. 1983. Transformation parameters and pp60src localization in cells infected with partial transformation mutants of Rous Sarcoma virus. Mol. Cell. Biol. 3:731–746.

Rovasio, R. A., A. Delouvee, K. M. Yamada, R. Timpl, and J. P. Thiery. 1983. Neural crest cell migration: requirements for exogenous fibronectin and high cell density. J. Cell Biol. 96:462–473.

Singer, I. I. 1979. The fibronexus: a transmembrane association of fibronectin-containing fibers and bundles of 5-nm microfilaments in hamster and human fibroblasts. Cell. 16:675–685.

Sutherland, A. E., P. G. Calarco, and C. H. Damsky. 1988. Expression and function of cell surface extracellular matrix receptors in mouse blastocyst attachment and outgrowth. J. Cell Biol. 106:1331–1348.

Strickland, S., and V. Mahdavi. 1978. The induction of differentiation in teratocarcinoma stem cells by retinoic acid. Cell. 15:393–403.

Tamkun, J. W., D. W. DeSimone, D. Fonda, R. S. Patel, C. Buck, A. F. Horvitz, and R. O. Hynes. 1986. Structure of integrin, a glycoprotein involved in the transmembrane linkage between fibronectin and actin. Cell. 46:271–282.

Wickus, G. G., P. E. Branton, and P. W. Robbins. 1974. Rous Sarcoma virus transformation of the chick cell surface In Control of Proliferation in Animal Cells. B. Clarkson and R. Baserga, editors. Cold Spring Harbor Laboratory, Cold Spring Harbor, NY. 541–546.