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

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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 (Chiquet 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)[1] 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.

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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 fluorescein-conjugated rabbit antihuman fibronectin (Jackson ImmunoResearch Laboratories, Avondale, PA) and a 1:20 dilution of rhodamine-conjugated phalloidin (Molecular Probes, Eugene, OR). Coverslips were then washed three times with PBS and twice with distilled water, and then mounted in gelvatol/PBS (Gelvatol was a generous gift from Monsanto Corp., St. Louis, MO and Air Products and Chemicals, Allentown, PA). Immunofluorescence for integrin was performed using a 1:10 dilution of IgG purified goat anti-gp140 (integrin) serum (Brown and Juliano, 1986; generous gift of Dr. P. Brown). Procedures were performed as above except the coverslips were fixed in 3.7% formalin for 10 min, and a 1:30 dilution of affinity-purified rhodamine-conjugated rabbit anti-goat serum (Cappel Laboratories, West Chester, PA) was used for the secondary reagent. The cell layers were observed with a Zeiss standard microscope equipped with epifluorescence illumination.

Metabolic Labeling

For experiments involving [^{35}S]methionine, cells were labeled 3 d after plating with 20 μCi/ml of L-[^{35}S]methionine (New England Nuclear, Boston, MA) for 16 h in a 1:1 mixture of normal and methionine-free DME (Gibco Laboratories, Grand Island, NY). For phosphorylation experiments, cells were labeled 4 d after plating with 200 μCi/ml of [^{32}P]orthophosphate (New England Nuclear) for 4 h in phosphate-free Dulbecco's MEM (Flow Laboratories, McLean, VA) in the presence of 50 μM sodium orthovanadate (Collett et al., 1984) (Sigma Chemical Co., St. Louis, MO).

Immunoprecipitations

For integrin immunoprecipitations, metabolically labeled cell layers containing ~2 × 10^7 cells were washed and scraped into PBS containing 2 mM PMSF (Sigma Chemical Co.) pelleted, and lysed on ice in 500 μl TNC buffer (Knudsen et al., 1981) (10 mM Tris, 0.5 mM $CaCl_2$, 0.5% NP-40, 2 mM PMSF, pH 7.4). Lysates were then clarified by centrifugation at 13,000 *g* for 10 min. The lysates were then aliquoted for equal cell numbers by electronic cell counting of replicate platings (Coulter Electronics, Hialeah, FL), diluted into 1 ml of 0.15 M NaCl, 20 mM Tris-HCl pH 7.5, 1 mM $CaCl_2$, 2 mM PMSF, 1% NP-40 (Patel and Lodish, 1987) and incubated with 25 μl of rabbit antimouse fibronectin receptor (Patel and Lodish, 1986; generous gift of Dr. V. Patel, Northwestern University, Evanston, IL) for 1 h with shaking at 4°C. Samples were then incubated for 30 min with 50 μl of Pansorbin (Calbiochem-Behring Corp., La Jolla, CA) that had been preincubated for 1 h with nonlabeled cell extract. The Pansorbin-bound material was washed twice with the same buffer as above, once with 400 mM NaCl, 5 mM EDTA, 50 mM Tris-HCl (pH 8.0), 2 mM PMSF, 1% NP-40, 0.1% SDS, 0.5% sodium deoxycholate, and once with 0.01 M Tris-HCl, pH 6.8. Talin immunoprecipitations were performed as above except the cell lysates were diluted into NET buffer (Hogan et al., 1981) (400 mM NaCl, 5 mM EDTA, 50 mM Tris, 1% NP-40, 2 mM PMSF, pH 8.0) and incubated with 4 μl of rabbit antimammalian talin (Burridge and Connell, 1983; the generous gift of Dr. K. Burridge, University of North Carolina, Chapel Hill, NC). 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, 10% glycerol, 2% SDS, 5% 2-mercaptoethanol [reduced samples only], 0.001% bromphenol blue), boiled for 3 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 [^{35}S]-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 electroblotted 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 (Blotto) (Johnson et al., 1984) in PBS for 30 min 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 1:1,000 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-1-naphthol 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, ^{32}P-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 hydrolysed 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., 1981; 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

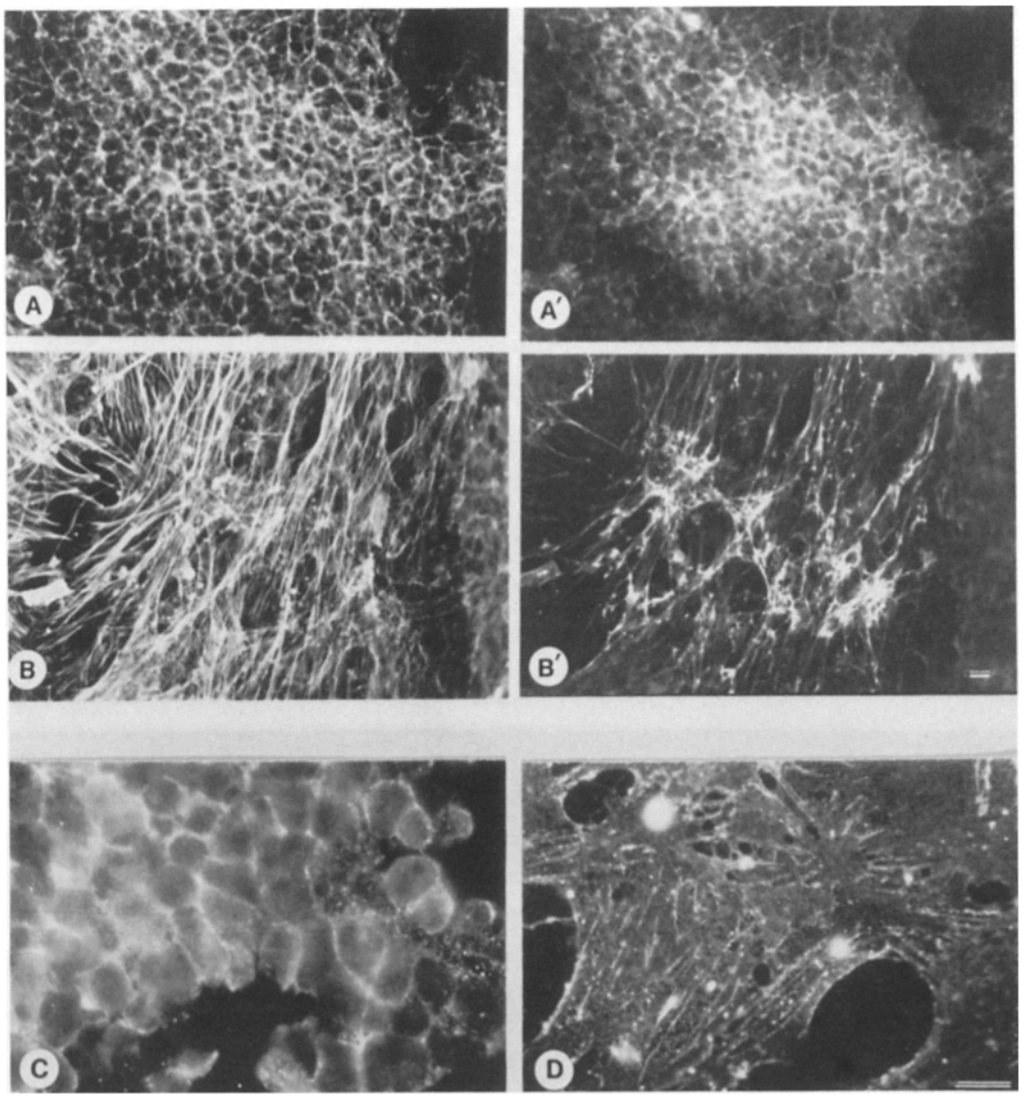

Figure 1. The localization of actin, fibronectin, and integrin on nontreated and treated F-9 teratocarcinoma cells. Nontreated cells (*A*, *A'*, and *C*) and treated cells (*B*, *B'*, and *D*) were grown on coverslips, fixed, and stained for fluorescence microscopy with either rhodamine-conjugated phalloidin (*A* and *B*) and fluorescein-conjugated goat antihuman fibronectin antiserum (*A'* and *B'*), or IgG-purified goat antiintegrin serum followed by affinity-purified rhodamine-conjugated rabbit antigoat serum (*C* and *D*) after 7 d of culture. Bars, 10 μm.

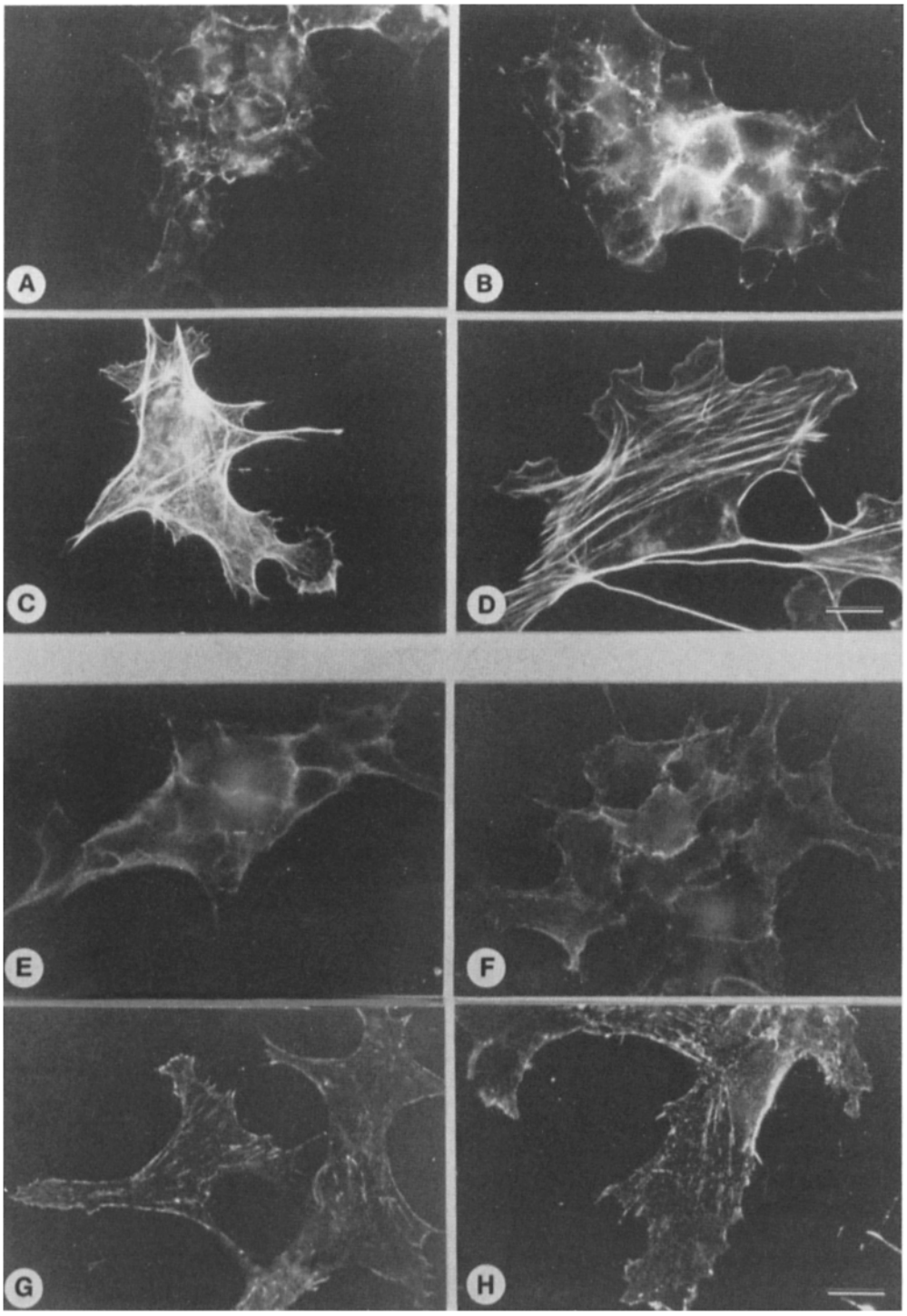

Figure 2. Localization of actin and integrin during the differentiation of treated F-9 cell cultures. Cells grown on coverslips in the presence of retinoic acid were fixed and stained for fluorescence microscopy with either rhodamine-conjugated phalloidin (*A*, *B*, *C*, and *D*) or IgG purified goat antiintegrin serum followed by affinity-purified rhodamine-conjugated rabbit antigoat serum (*E*, *F*, *G*, and *H*) on days 1 (*A* and *E*), 2 (*B* and *F*), 3 (*C* and *G*), and 4 (*D* and *H*) after plating. Bars, 10 μm.

3 d 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 d 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

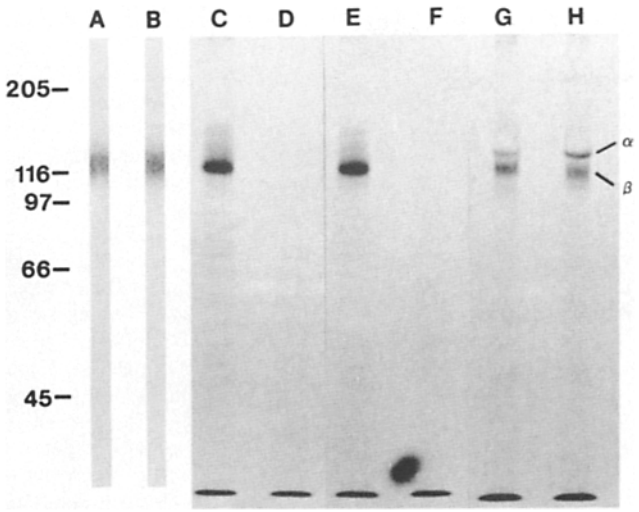

Figure 3. Immunoblots and immunoprecipitations of integrin from cell extracts of nontreated and treated F-9 cells. Data shown are blots or fluorographs of one-dimensional 7.5% SDS-polyacrylamide gels. Lanes *A* and *B* represent immunoblots of material from equal numbers of (*A*) nontreated and (*B*) 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.

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 (*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 complex after reduction with 2-mercaptoethanol. Lanes *G* and *H* represent nonreduced immunoprecipitates of (*G*) nontreated and (*H*) treated cell lysates and reveal the characteristic 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 [^{32}P]orthophosphate after 4 d of retinoic acid treatment, the time when fibronectin, actin, and integrin reorganization is becoming apparent. Fig. 4 represents data from this experiment. Lanes *A* and *B* depict total ^{32}P-labeled material from equal numbers of (*A*) nontreated and (*B*) treated cells and show a similar overall pattern of phosphorylation between the two cell types. Lanes *D*, *E*, and *G* represent ^{32}P-labeled material immunoprecipitated for integrin from equal numbers of (*D* and *G*) nontreated and (*E*) treated cells. Lanes *D* and *E* are reduced samples and lane *G* is nonreduced material. Lanes *C* and *F* are material immunoprecipitated from [^{35}S]methionine-labeled cells to serve as markers for (*C*) reduced and (*F*) nonreduced integrin. These data demonstrate that both the alpha and beta chains of the receptor complex are phosphorylated, and that the phosphorylation state of the integrin complex decreases dramatically during the differentiation of F-9 cells. Lanes *H–K* represent material immunoprecipitated for the cytoskeletal protein, talin, from equal numbers of [^{35}S]methionine (lanes *H* and *I*) and ^{32}P-labeled (lanes *J* and *K*) nontreated (*H* and *J*) and treated (*I* and *K*) F-9 cells. These data indicate that talin, another protein involved in the transmembrane-cytoskeleton linkage, does not undergo a decrease in phosphorylation after 4 d of retinoic acid treatment.

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, trophectoderm, is also accompanied by a change in integrin distribu-

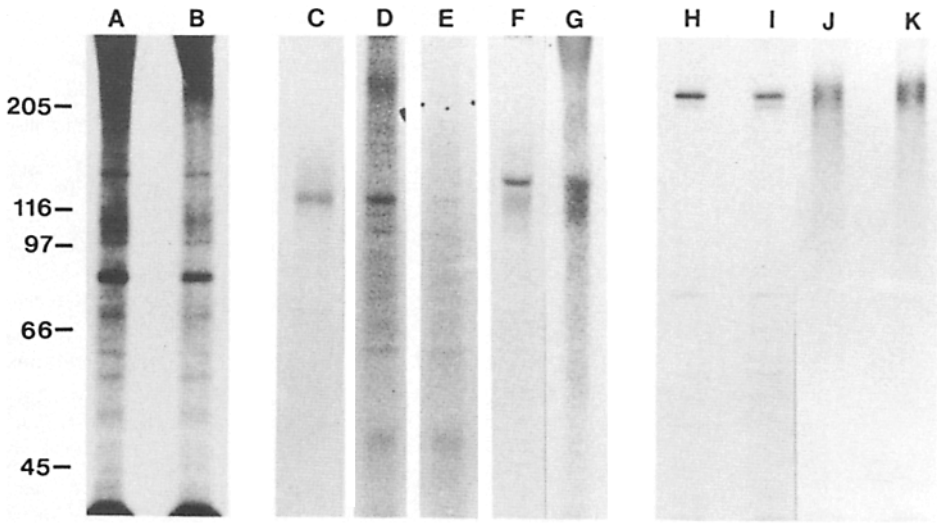

Figure 4. Integrin and talin phosphorylation. Lanes *A* and *B* are total [^{32}P]orthophosphate-labeled material from equal numbers of (*A*) nontreated and (*B*) treated cells after 4 d of culture. Lanes *D*, *E*, and *G* represent material immunoprecipitated from [^{32}P]orthophosphate labeled lysates 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 [^{35}S]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*) [^{35}S]methionine and (*J* and *K*) ^{32}P-labeled (*H* and *J*) nontreated and (*I* and *K*) treated F-9 cells.

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 Yahara, 1976; Wickus 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

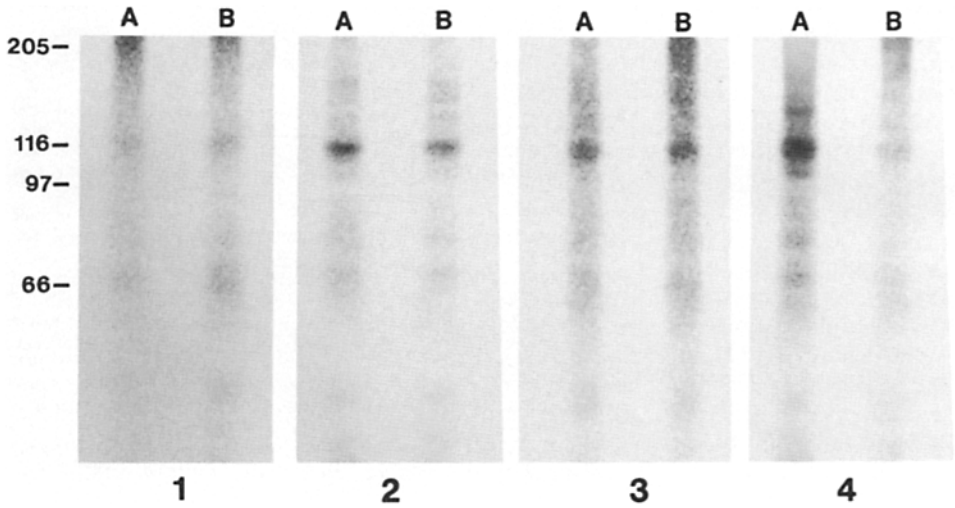

Figure 5. Integrin phosphorylation during the differentiation of F-9 cells. [^{32}P]Orthophosphate-labeled material was immunoprecipitated from (*A*) nontreated and (*B*) treated cells after 1 (*1*), 2 (*2*), 3 (*3*), and 4 (*4*) d of culture. Each pair of lanes contains material isolated from equal cell numbers. Note that the fainter bands at day 1 are due to the use of fewer numbers of cells at this early time.

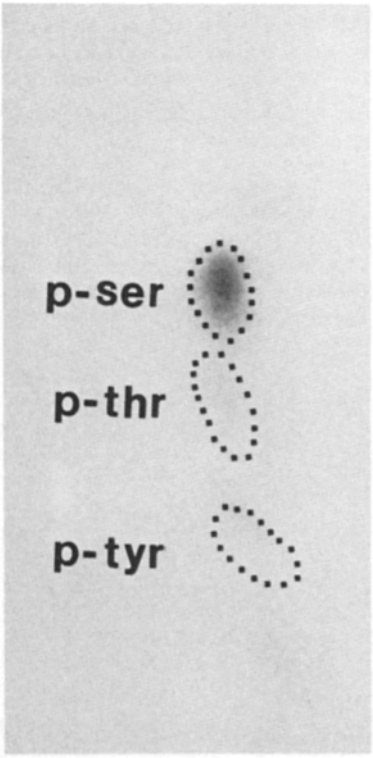

Figure 6. Phosphoamino acid analysis of integrin immunoprecipitated from nontreated F-9 cells. Immunoprecipitations of [^{32}P]orthophosphate-labeled cell lysates were processed for amino acid analysis and separated by thin-layer electrophoresis. Phosphoamino acid standards were visualized with ninhydrin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

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