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The Regulation of Growth and Intracellular Signaling by Integrins

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I. Introduction

INTEGRINS are a family of more than 20 different transmembrane receptors composed of noncovalently associated α- and β-subunit heterodimers (1). Twelve different α-subunits, each approximately 1000 residues in length, and eight different β-subunits, each approximately 750 residues, have been identified. The receptor consists of a very large extracellular domain, a transmembrane region, and a relatively short cytoplasmic region. The extracellular domain binds to various ligands including extracellular matrix (ECM) proteins, such as fibronectin (FN), vitronectin (VN), and collagen (Col), and to other cell surface receptors such as ICAM-1 (intercellular adhesion molecule) and VCAM-1 (vascular cell adhesion molecule). The receptor cytoplasmic domains interact with cytoskeletal proteins.

In addition to their role as adhesion receptors, integrins also function as signaling receptors and have been shown to regulate reorganization of the cytoskeleton, intracellular ion transport, lipid metabolism, kinase activation, and gene expression. In this review we will discuss the diverse integrin-mediated signals presently known, with emphasis on the integrin-mediated signals that regulate cell growth and survival. For a discussion of integrin-mediated regulation of cell migration, differentiation, and integrin-ligand binding, the reader is referred to other integrin reviews (2–6).

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II. Integrins, Cell Growth, and Cell Survival

The requirement of cell adhesion for growth in normal cells was first described by Stoker *et al.* (7), who found that normal cells were blocked in the G1 phase of the cell cycle when cultured in suspension. They termed this phenomenon "anchorage-dependence." With the discovery of the integrin family of receptors as the major ECM receptors, integrins were implicated as regulators of cell growth.

Some of the first work directly linking integrins to growth regulation came from studies of T cell activation. T cells are induced to proliferate by activation of the T cell receptor complex (TCR) (8). Using antibodies against LFA-1 (integrin $\alpha_L\beta_2$), van Noesel *et al.* (8) showed that different antibodies could either enhance or inhibit TCR-induced proliferation. Subsequent work demonstrated that costimulation of both integrins and the TCR could induce T cell proliferation under conditions in which stimulation of either receptor alone was not sufficient (9–14). These results indicate that integrin signals can regulate T cell proliferation and also suggest that integrins synergize with other signaling receptors. In addition to regulating T cell proliferation, integrins have been found to control proliferation in nonlymphoid cells such as endothelial cells (15, 16), hepatocytes (17), and fibroblasts (18, 19).

Although integrin-dependent signals are required for cell growth, accumulating evidence indicates that under certain conditions integrins also suppress growth. Giancotti and Ruoslahti (20) were the first to report that transformed cells that overexpress integrin $\alpha_5\beta_1$ fail to grow when suspended in soft agar, in contrast to wild type cells, but grow normally when adherent. This growth inhibition correlated with reduced tumorigenicity *in vivo*. A similar effect of integrin overexpression was reported in studies using K562 erythroleukemia cells and HT29 colon carcinoma cells (21, 22). Another example of growth suppression by integrins was reported by Meredith *et al.* who found that the alternatively spliced integrin β_{1C} inhibited cell cycle progression when transiently expressed in fibroblasts (23). β_{1C} had a similar effect when expressed in Chinese hamster ovary (CHO) cells (24). In addition, expression of the integrin β_4 subunit in rectal carcinoma cells will induce a partial G1 arrest (25).

In addition to regulating cell growth, integrins are also involved in the regulation of cell survival or programmed cell death (PCD). PCD, or apoptosis, is the process whereby cells are induced to activate their own death. PCD occurs in a wide variety of cell types and is required for the development of many tissues (26). Recent evidence indicates that maintaining

cells in suspension, in the absence of adhesion to the ECM, will induce PCD (27–29). This effect is dependent on integrin ligation. Human umbilical vein endothelial cells (HUVECs), when cultured in suspension, rapidly die and display all of the characteristics of PCD (27, 29). Ligation of the β_1 integrins, but not other cell surface receptors, is sufficient to rescue these cells from PCD, suggesting that integrins provide a survival signal (27). Similar results have been obtained in other systems. For example, endothelial cells are dependent on $\alpha_v\beta_3$ signaling for survival *in vivo* (30), CID-9 mammary epithelial cells require β_1 integrin ligation (31), and LIM 1863 colon carcinoma cells are dependent on α_v integrin ligation (32). These results establish the importance of integrin ligation for cell survival and indicate that different integrin receptors have the capacity to function as survival receptors.

The mechanisms whereby integrins regulate cell growth and PCD are not clear; different integrin-mediated signals may be involved. In the following sections, we will discuss the various integrin-mediated signals and how they can regulate cell growth and cell survival.

III. Integrin Signaling

A. Effects on the cytoskeleton

Integrins are transmembrane proteins that attach the cell to the ECM and anchor the cytoskeleton to the plasma membrane (1, 33). This dual role confers to integrins the unique ability to transduce information about the cell's external environment into structural changes within the cell. The binding of integrins to the ECM initiates both the localization of cytoskeletal proteins into structures known as focal adhesions and the assembly of actin microfilaments (33). This integrin-mediated reorganization of cytoskeletal proteins and actin is the basis for cell spreading and migration.

1. Integrin β-cytoplasmic domain. Although both the α- and β-integrin subunits contain cytoplasmic domains, it is primarily the integrin β-cytoplasmic domain that appears to be required for cytoskeletal interactions. *In vitro*, the β_1-cytoplasmic domain has been shown to bind directly to the cytoskeletal proteins α-actinin and talin (34, 35). A peptide containing residues 780–789 of β_1 can bind to talin (36). Peptides containing either residues 768–777 or 785–794 bind directly to α-actinin (35, 37) (summarized in Fig. 1). Whether all of these interactions occur *in vivo* is not known.

Studies *in vivo* have tested the ability of transfected integrin mutants and chimeras to colocalize with endogenous integrins and cytoskeletal proteins in focal adhesions. Focal adhesions are regions of tight association between the plasma membrane and the ECM (10–15 nm) and contain high concentrations of many proteins including components of the cytoskeleton, actin microfilaments, signaling molecules, and integrins (33). Focal adhesions are formed in part by integrin-mediated signals (see below) and are thought to play a key role in triggering signal transduction pathways and in regulating the cytoskeleton.

All of the information necessary for integrin localization to focal adhesions is present in the β-cytoplasmic domain. Both chimeras containing the β_1-cytoplasmic domain and receptors expressing α-subunit cytoplasmic domain truncations are localized to focal adhesions (38–41). Results from deletion studies indicate that sequences near the C terminus of β_1 are required (42, 43) (see Fig. 1). By screening different point mutants, Reszka *et al.* (44) identified three clusters of amino acids which, when mutated, impair β_1 localization (Fig. 1). Two of these clusters share the amino acid motif "NPXY." Interestingly, one of these NPXY motifs is required for β3-mediated melanoma cell migration (45), a process dependent on integrin-mediated regulation of the cytoskeleton.

Other studies *in vivo* have investigated the effect of clustering integrins with microbeads coated with antiintegrin antibodies or ECM substrates. Clustering integrins with these coated microbeads will induce the colocalization of many focal adhesion proteins (46–50). This effect is probably very similar to the colocalization that occurs during focal adhesion formation. Lewis and Schwartz (46) found that clustering integrins induced the colocalization of the focal adhesion proteins talin, α-actinin, the focal adhesion kinase (FAK), and F actin. By screening β_1-cytoplasmic domain deletion mutants, they found that colocalization of talin, FAK, and F-actin was dependent on residues 791–799 of the β_1-cytoplasmic domain (Fig. 1) (46). As mentioned above, Tapley *et al.* (36) found that residues 780–789 were required for binding of β_1-cytoplasmic peptides to talin *in vitro*. Taken together, these results suggest that both regions contain information nec-

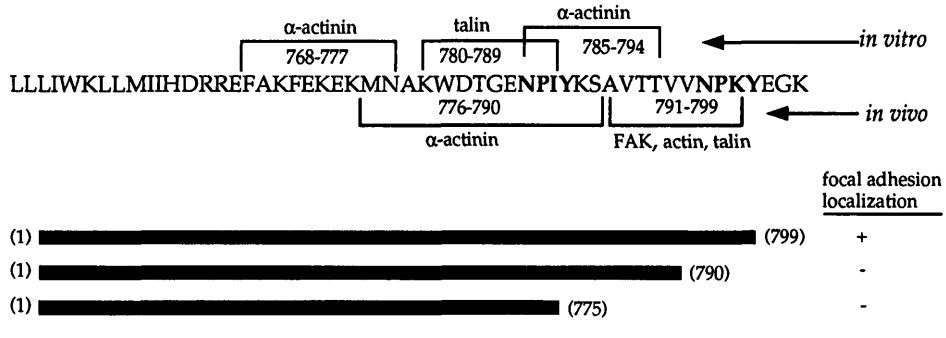

FIG. 1. Functional domains in the β_1-cytoplasmic tail. *In vitro*, peptides comprising amino acids 768–777 and 785–794 of the β_1-tail bind to α-actinin while a peptide composed of amino acids 780–789 can bind talin. *In vivo*, the residues important for colocalization of α-actinin, FAK, actin, and talin with theβ_1 cytoplasmic tail were determined using microbeads coated with anti-β_1 antibody and cells expressing either wild type or mutated β_1-cytoplasmic tails. Deletions of residues 791–803 and 776–803 reduce focal adhesion localization of the β_1-integrin. (1) indicates the N terminus of β_1. The two NPXY sequences involved in β_1-focal adhesion localization are highlighted in *bold*.

essary for talin binding. Colocalization of α-actinin required residues 776–790 of the β_1-cytoplasmic domain, generally consistent with the *in vitro* data mentioned above (see Fig. 1) (46). Interestingly, colocalization of α-actinin, in the absence of talin and FAK, was not sufficient to promote localization of actin. This result is surprising given that α-actinin can bind to actin (51). One possible explanation is that the binding of α-actinin to actin may be regulated and require signals normally generated by the integrin sequences deleted. This regulation may be important for the formation and maintenance of focal adhesions as discussed below.

The importance of the structure of the β-subunit cytoplasmic domain is also demonstrated by the high conservation observed between species for each subtype (52, 53) and the conservation of sequence motifs found between the different β-subtypes (52). These motifs include the NPXY clusters discussed above. In contrast, integrin β-subtypes with divergent sequences, such as β_4, β_5, and β_{1C} or β_{1B}, do not normally participate in the formation of focal adhesions (23, 54, 55). The β_5-subunit contains a variant region within its cytoplasmic domain and does not readily localize to focal adhesions (56, 57). β_{1B} and β_{1C} are splice variants of β_1 that diverge in the 21 COOH-terminal residues of the cytoplasmic domain and also do not localize to focal contacts (23, 58), consistent with the results of studies using the β_1-deletion mutants. The β_4-cytoplasmic domain is completely unrelated to other β-subunits and appears to be linked to intermediate filaments rather than the actin cytoskeleton (54, 59–61).

2. *Cell spreading and the formation of focal adhesions.* Integrins regulate adhesion and spreading of cells on ECM substrates. Integrin-mediated cell spreading is dependent on the β-cytoplasmic domain. By using mouse fibroblasts expressing transfected chicken β_1-subunits, Guan *et al.* (62) found that deletion of C-terminal residues from the β_1-cytoplasmic domain was sufficient to block spreading of transfectants on antibodies against the extracellular domain of chicken β_1 (62). Deletion of the β_1-cytoplasmic domain also blocked cell spreading on ECM substrates (42). In contrast, the α-cytoplasmic domain does not appear to be required for spreading. Using cells that express mutants of the integrin $\alpha_{IIb}\beta_3$ (fibrinogen receptor), Ylanne *et al.* (41) found that deletion of the β_3-cytoplasmic domain blocked cell spreading on fibrinogen, while deletion of the α_{IIb}-cytoplasmic domain had no effect. In addition, truncation of the α_5-subunit cytoplasmic domain had little or no effect on spreading of CHO cells expressing $\alpha_5\beta_1$ on FN (40).

Miyamoto *et al.* (47, 48) have studied the effects of integrin-ligand binding on the ability of the β_1-cytoplasmic domain to induce colocalization of various proteins. They found that the integrin $\alpha_5\beta_1$ clustered with inhibitory antibodies induced colocalization of actin, talin, vinculin, α-actinin, FAK, and tensin. Similar results were obtained when $\alpha_5\beta_1$ was clustered using FN-coated beads. In contrast, when $\alpha_5\beta_1$ was clustered with noninhibitory antibodies, only FAK and tensin were colocalized. However, clustering $\alpha_5\beta_1$ with noninhibitory antibodies in the presence of the monomeric ligand RGD (arginine-glycine-aspartic acid) restored colocalization of all the proteins. RGD peptides have also been shown to induce the recruitment of one integrin ($\alpha_5\beta_1$) to focal adhesions formed by another integrin ($\alpha_2\beta_1$) (39). These results, together with the fact that the β-cytoplasmic domain is required for focal adhesion localization and cytoskeletal protein binding, suggest a model in which ligand binding leads to unmasking of the β-cytoplasmic domain. Unmasking of the β-cytoplasmic domain would then generate the signals necessary for cell spreading and focal adhesion formation. This model also suggests that the α-cytoplasmic domain may function to block β-cytoplasmic domain signals in the absence of ligand binding.

In addition to cytoskeletal protein interactions, other integrin-mediated signals are also required for cell spreading and focal adhesion formation. As will be discussed in detail below, integrins induce many signaling events including activation of proteins such as the small GTPase Rho (63), phospholipase A_2 (PLA_2) (64, 65), protein tyrosine kinases (62, 66–69), and protein kinase C (PKC) (65, 70). Inhibition of PLA_2 or PKC prevents cell spreading (64, 65, 70), and inhibition of Rho or tyrosine kinases blocks formation of stress fibers and focal adhesions (71–73). In addition, the interaction between α-actinin and actin is sensitive to the lipid phosphatidylinositol bisphosphate (PIP_2) (74), whose levels have been found to depend upon integrin-mediated cell adhesion (see below) (63, 75).

Integrins associate with actin via a potentially complex array of indirect linkages (see Fig. 2). As mentioned above, integrins have been shown to bind to both α-actinin and talin. Each of these proteins can bind actin. In addition, talin can bind to actin indirectly through a vinculin-α-actinin link (51, 76) or through a vinculin-tensin link (77). These alternative linkages may play a role in the regulation of focal adhesions. Indeed, microinjection studies have suggested that talin participates in the initial formation of focal adhesions, whereas α-actinin is more important for their maintenance (78, 79). These indirect linkages may provide many opportunities for regulatory fine-tuning.

3. *Cell spreading, growth, and survival.* Accumulating evidence indicates that integrin-mediated cell spreading is linked to growth control in normal cells. By accurately controlling the extent of cell spreading, Folkman and Moscona (15) found that cell spreading was directly proportional to cell growth. This effect of spreading appeared to be due to the regulation of cellular sensitivity to growth factors, implying synergy between integrin-mediated spreading and growth factor receptor signaling (80). Integrin clustering alone was not sufficient to promote growth; however, integrin clustering in the absence of spreading was sufficient to induce expression of early genes (junB and Ras) involved in the G0/G1 transition (17). These results, together with the observation that cells in suspension are arrested just before G1/S (81), suggest that integrins may act at two points in the cell cycle: regulation of the G0/G1 transition by ligand binding and regulation of G1/S by cell spreading.

Plopper *et al.* (50) have isolated focal adhesions and found that, in addition to integrins and cytoskeletal proteins, focal adhesions also contain many proteins implicated in growth control. These include growth factor receptors and signaling molecules such as c-*src*, FAK, phosphoinositol 3-kinase (PI

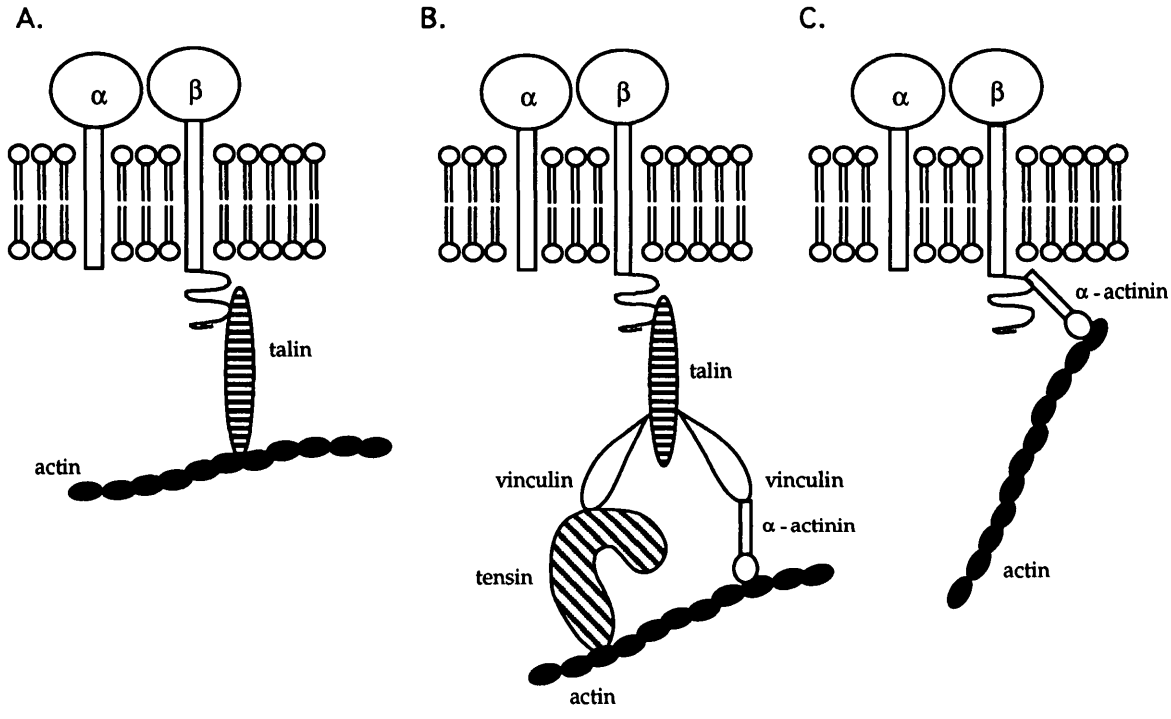

FIG. 2. Schematic representation of three potential linkages from the integrin β-subunit cytoplasmic domain to actin microfilaments. *In vitro* studies suggest the existence of different protein connections within focal adhesions. In panel A, talin binds directly to both the β-subunit and to F actin (polymerized actin). In panel B, talin again binds directly to the β-subunit but also binds to vinculin. Vinculin binds to either tensin or to α-actinin, which in turn binds to F actin. In panel C, α-actinin binds directly to both the β-subunit and to F actin; talin is not involved. Each of these three cases may exist within cells simultaneously, sequentially, or not at all. *Circles* containing "α" and "β" represent integrin α- and β-subunits, respectively.

3-kinase), and phospholipase Cγ (PLCγ). Substrates for growth factor-regulated pathways have also been found in focal adhesions including tensin, paxillin, and p130cas (82–84). Miyamoto *et al.* (48) found that 20 signal transduction molecules, including RhoA, Rac1, Ras, Raf, the mitogen-activated protein kinase (MAP kinase), MAP kinase kinase (MEK), and the *jun* kinase (JNK) were colocalized with clustered integrins, suggesting that they might also be localized in focal adhesions, although this has not been demonstrated. These observations imply that integrins may regulate cell proliferation by inducing the colocalization of signaling molecules into a signaling complex, thereby facilitating the interactions of these proteins.

Integrins can also physically interact with growth factor receptor substrates. One example is the insulin receptor substrate. The insulin receptor substrate 1 (IRS-1) is a 180-kDa protein that is tyrosine phosphorylated by the insulin receptor in response to insulin stimulation and is required for insulin receptor signaling (85). Vuori and Ruoslahti (86) found that insulin triggers the association of IRS-1 with the integrin $\alpha_v\beta_3$ (the VN receptor). This association correlated with a 2.5-fold increase in proliferation of insulin-treated cells when they were plated on VN relative to other substrates (86).

In addition to effects on growth, integrin-mediated cell spreading may also be linked to the regulation of cell survival. Re *et al.* (29) found that the extent of cell survival in endothelial cells was directly proportional to the extent of cell spreading on either FN or VN. They suggest that a critical threshold of spreading is required to suppress PCD. Clustering integrins, in the absence of spreading, was not sufficient to rescue cells in suspension (29). However, cell spreading does not appear to be critical in all cell types. Blocking β_1-integrins of CHO cells and CID-9 mammary epithelials is sufficient to trigger PCD in the absence of any changes in cell spreading (31, 87). Perhaps in these cases integrin-ligand binding and/or clustering alone is required for survival.

B. Generation of second messengers

1. Intracellular ions. In addition to providing a direct link between the ECM and the cytoskeleton, integrins have also been shown to regulate the production of second messengers within the cell. For example, integrins have been shown to regulate intracellular H+ concentrations (pH) via activation of the Na+/H+ antiporter (88–90). Integrins have also been shown to trigger a rise in intracellular free calcium ion concentration ($[Ca^{2+}]_i$). $[Ca^{2+}]_i$ functions as a second messenger by regulating a variety of protein kinases, phosphatases, and other enzymes (91). In endothelial cells the integrin-mediated elevation in $[Ca^{2+}]_i$ is dependent on ligation of α_v-integrins but not other integrins (92). This $[Ca^{2+}]_i$ transient is also dependent on a 50-kDa integrin-associated protein, which may function as a calcium channel (93). $\alpha_v\beta_3$ also induces $[Ca^{2+}]_i$ transients in osteoclasts (94). β_2-Integrins can induce an increase in $[Ca^{2+}]_i$ by both influx and mobilization of intracellular calcium stores (95–97).

While regulation of the antiporter appears to be a general property of integrin signaling, not all integrin receptors have the

capacity to regulate $[Ca^{2+}]_i$. For example, whereas the integrins $\alpha_2\beta_1$, $\alpha_5\beta_1$, and $\alpha_v\beta_3$ all activate the antiporter in endothelial cells, only $\alpha_v\beta_3$ can trigger a $[Ca^{2+}]_i$ transient (92, 98). These observations demonstrate that the regulation of intracellular pH and $[Ca^{2+}]_i$ depend on two separate signaling pathways. Moreover, these observations suggest that different receptors within the same cell can generate unique signals.

Integrins also regulate K^+ influxes in neuronal cells. Arcangeli *et al.* (99) found that a potassium channel in neuroblastoma cells is required for hyperpolarization and neurite outgrowth in response to integrin signaling (99). Integrin-mediated regulation of the potassium channel is dependent on a pertussis toxin-sensitive G protein.

2. *Lipids.* Integrins regulate the metabolism of inositol phospholipids. The first clue to this came from results demonstrating the synergy between growth factors and cell adhesion. In fibroblasts, the platelet-derived growth factor (PDGF) can stimulate the Na^+/H^+ antiporter in cells attached to FN, but not in unattached cells (100). This stimulation was found to be PKC-dependent. Calcium mobilization triggered by PDGF was also found to be adhesion-dependent (101). Both PDGF-mediated PKC activation and Ca^{2+} mobilization depend on the PIP_2 breakdown products inositol triphosphate (IP_3) and diacylglycerol (DAG), respectively (102). Hydrolysis of PIP_2 is induced by the PDGF-mediated activation of PLC (103–105). McNamee *et al.* (75) found that cell adhesion stimulated a quick increase in the rate of synthesis and the absolute level of PIP_2. In contrast, detaching cells caused a dramatic decrease in PIP_2 levels (75). Together, these data indicate that integrins regulate the levels of substrate available for hydrolysis by growth factor receptor-activated PLC.

Integrins appear to regulate the levels of PIP_2 by activation of a phosphatidylinositol 4-phosphate 5-kinase (PIP 5-kinase) (75). Recently, Chong *et al.* (63) reported that the small GTPase Rho activates a PIP 5-kinase. The Rho family of small GTPases, Rho, Rac, and Cdc42, have been implicated in the regulation of actin filament organization and focal contact formation (106). Rho alone regulates the assembly of actin stress fibers and focal adhesions induced by serum (107). The mechanism of integrin-induced PIP 5-kinase activation is unclear; however, several lines of evidence suggest that Rho may be involved. First, when Rho is inactivated in adherent cells, PIP_2 levels decrease (63); second, PDGF can induce Ca^{2+} mobilization in round cells microinjected with an activated variant of Rho (63). Integrins might regulate Rho by activating a Rho-specific exchange factor or GTPase-activating protein. Interestingly, another Rho family member, Cdc42, is implicated in integrin $\alpha_{IIb}\beta_3$ signaling in platelets. Clustering $\alpha_{IIb}\beta_3$ enhanced translocation of Cdc42 to the cytoskeleton, accompanied by protein tyrosine phosphorylation and actin polymerization (108). Whether Cdc42 regulates PIP 5-kinase activity is not clear.

In addition to PIP 5-kinase, phosphoinositol 3-kinase (PI 3-kinase), which phosphorylates PI, 4-PIP, and 4,5-PIP_2 to generate 3-PIP, 3,4-PIP_2, and 3,4,5-PIP_3, respectively, is also implicated in integrin signal transduction. In osteoclasts, osteopontin binding to the integrin $\alpha_v\beta_3$ stimulated PI 3-kinase activity, and PI 3-kinase was found to coimmunoprecipitate with $\alpha_v\beta_3$ (109). In addition, PI 3-kinase was found to coimmunoprecipitate with tyrosine-phosphorylated FAK in response to cell adhesion (110, 111). PI 3-kinase was also tyrosine phosphorylated upon cell adhesion and found to be phosphorylated by FAK *in vitro* (110, 111). These results suggest that FAK may mediate integrin-induced PI 3-kinase activation.

In addition to phosphoinositides, arachidonic acid metabolism is also regulated by integrin-mediated signals. Plating HeLa cells onto Col or immobilized RGD peptide triggered the sequential activation of PLA_2, release of arachidonic acid, formation of lipoxygenase metabolite(s), production of DAG, activation of PKC, and the induction of cell spreading (64, 65). These effects depend on the integrin β_1 (112). In addition, integrins can also regulate PLCγ. Adhesion of rat epithelial cells to Col stimulated production of DAG, which also required β_1-integrins (113). In T cells, clustering β_2-integrins induced tyrosine phosphorylation of PLCγ and correlated with integrin-mediated Ca^{2+} mobilization (114). Therefore, integrins are capable of activating both lipid kinases (PI 3-kinase and PI 5-kinase) and phospholipases (PLA_2 and PLC), suggesting that lipids may function as key mediators of integrin-induced signals.

C. Activation of protein kinases

1. *FAK.* Protein tyrosine kinases play an important role in the control of numerous cellular functions such as cell growth and differentiation (115). A potential role of tyrosine phosphorylation in integrin-mediated signaling was originally suggested from studies showing that phosphotyrosine-containing proteins are greatly enriched in focal adhesions (68, 116, 117). Recent evidence indicates that upon integrin-ligand binding a number of proteins are tyrosine phosphorylated (67, 68, 118), including the focal adhesion proteins paxillin (68), tensin (119), and FAK (68, 69, 119, 120).

FAK represents a new family of nonmyristylated, tyrosine kinases (molecular radius of 125 kDa). FAK was first identified as a phosphotyrosine protein in chicken embryo fibroblasts transformed with v-*src* (33, 69, 82, 121). FAK is highly conserved among amphibians (122, 123), birds (69), rodents (82), and man (124–126). FAK-deficient mice, generated by targeted gene disruption, are embryonic lethal and display a general defect in mesoderm development (127) (see below).

FAK is structurally distinct from other known tyrosine kinases. Its central catalytic domain is flanked by large N-terminal and C-terminal domains that lack any significant homology with other protein tyrosine kinases (69, 82). FAK does not possess any known determinants for membrane association, *src* homology 2 (SH2), or *src* homology 3 (SH3) domains but does contain SH3-binding sequences and potential SH2-binding sequences. The N terminus of FAK binds *in vitro* to peptides from the membrane-proximal region of the β_1-integrin cytoplasmic domain (128). Whether this interaction occurs *in vivo* is not known. Targeting of FAK to focal adhesions is dependent on a focal adhesion targeting sequence located in the distal part of the C terminus of FAK (AA 904-1040). This focal adhesion targeting sequence is required for FAK localization and will induce focal adhesion targeting when linked to other, unrelated proteins (129, 130).

FAK phosphorylation is induced by attachment of various

cell lines (NIH3T3, BALB/c 3T3, KB carcinoma cells) to FN and to other ECM proteins such as laminin (Lam), Col, and VN but not to nonspecific ligands such as poly-L-lysine (68, 82, 118, 120). Soluble FN has no effect (120). Clustering of β_1- and β_3-integrins also induced FAK phosphorylation (62, 67). Studies using cytochalasin D, which selectively disrupts the network of actin filaments, show that the integrity of the actin cytoskeleton is required for increased phosphorylation of FAK (68).

In platelets, thrombin or Col increased tyrosine phosphorylation of FAK, and this phosphorylation required platelet aggregation mediated by the binding of fibrinogen to GpIIb/IIIa (integrin aIIbb3) (131). FAK was not activated after thrombin stimulation of Glanzmann's thrombasthenic platelets (platelets deficient in the fibrinogen receptor, the integrin $\alpha_{IIb}\beta_3$), suggesting that FAK is indeed functionally linked to integrins and that, in the case of platelets, it might also play a role in platelet activation and/or aggregation (131).

Mutational analyses of the integrin β_1-subunit showed that FAK phosphorylation depends on the cytoplasmic domain (62, 132). These findings are further supported by experiments using a series of chimeric human integrin-interleukin 2 receptors transiently expressed in fibroblasts (132). Expression and clustering of the cytoplasmic domains of the β_1-, β_3-, and β_5- integrins, in the absence of their transmembrane and extracellular domains, were sufficient to induce FAK phosphorylation.

Recently Calalb *et al.* (133) demonstrated that FAK is tyrosine phosphorylated on at least four identified sites in an adhesion-dependent manner: Tyr 397, Tyr 407, Tyr 576, and Tyr 577. A fifth site, Tyr 925, was identified by Schlaepfer *et al.* (134). Of these residues, Tyr 397 is the major site of autophosphorylation (135, 136). In addition to a possible regulatory function, phosphorylation of Tyr 397 also creates a high affinity binding site for the SH2-domain containing proteins Src, Fyn (137), and PI 3-kinase (110). c-*src* Phosphorylates FAK on Tyr 925, which can then function as a binding site for the SH2-domain of the Grb2 adapter protein (134). Since Grb2 is constitutively associated with the Ras GDP/GTP exchange factor Sos, integrin-mediated FAK activation may therefore be linked to activation of the Ras pathway (134).

In both platelets and fibroblasts, integrin-stimulated FAK tyrosine phosphorylation correlates with an increase in the intrinsic kinase activity of FAK (118, 131). Some potential FAK substrates have been identified. The cytoskeletal protein paxillin has been shown to be phosphorylated by FAK both *in vitro* (138) and *in vivo* (139). Interestingly, phosphorylation of paxillin on tyrosine creates SH2-domain-binding sites for the adapter protein Crk, the Src-regulatory kinase Csk, and the Src kinase (139). As mentioned above, PI 3-kinase is phosphorylated by FAK *in vitro* (110), though the consequences of this tyrosine phosphorylation are unknown.

Recent data indicate that FAK plays a role in cell motility. Embryonic mesodermal cells isolated from mouse embryos in which FAK was deleted by homologous recombination were less well spread than FAK-positive control cells but were able to form stress fibers terminating in apparently normal focal adhesions (127). In fact, the FAK-deficient cells exhibited even more focal adhesions than FAK-positive cells as well as an increased number of microspikes. Surprisingly, many focal adhesion proteins, including paxillin, were phosphorylated. The FAK-deficient mesodermal cells had reduced mobility *in vitro*. It was proposed that absence of FAK resulted in tighter contact formation of cells with the substrate, possibly because of reduced turnover of focal adhesions. The requirement of FAK for cell motility may also explain the abnormal development of the head mesenchyme, lateral mesoderm, extraembryonic mesoderm, heart, and vasculature in the homozygous null mutant embryo (140). These abnormalities were not due to any defects in cell proliferation and/or differentiation (141).

While deletion of FAK inhibits motility, overexpression of FAK appears to correlate with increased motility. Highly motile melanoma cells exhibit higher expression of FAK in cell culture (142). Increased levels of FAK expression have also been correlated with the invasive and metastatic phenotype in solid tumors (142–144). These data indicate that overexpression or perhaps activation of FAK plays a role in cell locomotion and invasiveness. Such an effect would be consistent with the ability of FAK to modulate assembly/disassembly of focal contacts and actin filaments. These findings are also consistent with earlier observations, in which a role for FAK in cellular transformation events was suggested by the finding that FAK phosphorylation is increased in v-*src*-transformed cells (118, 121).

In addition to its activation by integrins, FAK is also activated by several growth factors. FAK phosphorylation has been shown to be stimulated by the mitogenic neuropeptides bombesin, vasopressin, and endothelin (145, 146). And more recently, FAK phosphorylation was shown to be modulated by PDGF and lysophosphatidic acid, a phospholipid that elicits a wide variety of cellular responses (147). The observation that FAK can be activated by both integrins and growth factor receptors indicates that FAK may be a point of convergence of these two signaling pathways in the regulation of cell migration.

2. *MAP kinase.* Growth factor receptors induce changes in gene expression and modulate cell growth by activating complex signal transduction cascades. Early events in these pathways include autophosphorylation of the growth factor receptor, stimulation of phospholipid turnover, and activation of ser/thr protein kinases such as PKC and the MAP kinase family (115, 148–150). MAP kinases, also known as ERKs (extracellular-regulated kinases), are activated by phosphorylation on both tyrosine (Tyr 185) and threonine (Thr 183) residues (151–153). Two forms of MAP kinase have been isolated from fibroblasts, referred to as either p42 or p44 MAP kinase (154), both of which become highly phosphorylated upon mitogenic stimulation (155). MAP kinases are phosphorylated by a single tyr/thr kinase MEK (156). MEK, in turn, is activated by phosphorylation on ser/thr residues by either Raf, MEK kinase (MEKK), or Mos (157–159). Activation of MEK by Raf kinase links MAP kinase to the Ras signal transduction cascade.

One of the striking features of MAP kinase is that its activation leads to its translocation from the cytoplasm to the nucleus (160, 161). As a result of this translocation, many substrates of MAP kinase include transcription factors, such as TCF, *jun*, *fos*, *myc* NF-IL6, TAL1, and ATF2 (149, 162). Thus,

MAP kinase may be a key molecule in the transmission of extracellular signals into the nucleus.

Recently, integrins have been shown to activate MAP kinases (48, 163–165). Integrin-mediated cell adhesion has been associated with activation of both the p42 and p44 MAP kinases (163–165), as well as their translocation into the nucleus (163). Activation of MAP kinase was observed when cells adhered to either FN, Lam, Col, or RGD-containing peptides but not when cells adhered to poly-L-lysine. These results suggest that multiple integrins can activate the kinases. Clustering of β_1-integrins is sufficient to induce MAP kinase activation and has also been shown to activate another MAP kinase family member, JNK (48, 164).

The pathway by which integrins mediate activation of MAP kinases remains unknown, but there are several possibilities. For example, components of the Ras pathway may be involved. Adhesion of 3T3 cells to FN promotes association of the adapter protein Grb2 with FAK, and cytochalasin D blocks both integrin-mediated FAK phosphorylation and MAP kinase activation (131). PI 3-kinase, which has been linked to integrin-dependent FAK activation, can also mediate MAP kinase activation. Another possibility is through the interaction of IRS-1 with the cytoplasmic domain of the integrin $\alpha_v\beta_3$ (86, 166). Vuori and Ruoslahti (86) found that insulin promotes the association of $\alpha_v\beta_3$ with IRS-1 and with Grb2 and Sos. One report suggests that the binding of certain antibodies against $\alpha_2\beta_1$ can activate p21 Ras (167). PKC might also be involved since it can directly phosphorylate Raf (150) and is activated upon cell adhesion to FN (65, 70). Which of these pathways is required for the activation of MAP kinases remains to be determined.

The activation of MAP kinases by growth factors and integrins appears to be quantitatively different. Integrin-mediated activation is slower yet persists longer than growth factor receptor-mediated activation (165). This dual mode of activation may be required for stimulation of cell growth. Like FAK, as discussed above, MAP kinases may also act as a point of convergence between integrin-mediated signaling and growth factor receptor signaling.

Recently, a novel 59-kDa ser/thr kinase was isolated using a yeast two-hybrid screen to identify proteins that interact with the β_1-cytoplasmic domain (168). This integrin-linked kinase (ILK) coimmunoprecipitated with integrin β_1 and was found to phosphorylate a β_1-cytoplasmic domain peptide *in vitro*. Interestingly, ILK kinase was reduced in response to FN, and overexpression of ILK inhibited integrin-mediated adhesion. These results suggest that ILK may be a proximal player in the regulation of integrin-controlled signals.

D. Induction of gene expression

Induction of gene expression by integrins has been studied in several cell types using a variety of integrin ligands (see Table 1) (2, 6). Integrins stimulate gene expression to regulate proliferation, differentiation, and matrix remodeling (2, 6, 169). Fibroblasts, epithelial cells, and monocytes have all been used as model systems by which to study gene expression (2, 6, 17, 169–174).

The control of gene expression by integrins depends on the cell type and the specific ECM proteins to which integrins bind. In fibroblasts, Col and FN induce the expression of metalloproteinases (MMPs) (2, 6). FN suppresses and enhances collagenase and gelatinase expression through $\alpha_4\beta_1$ and $\alpha_5\beta_1$, respectively (175). Plating-suspended fibroblasts on FN will also induce c-*fos* and c-*myc* expression (176). By using osteosarcoma cell lines expressing one of two Col receptor subtypes ($\alpha_1\beta_1$ or $\alpha_2\beta_1$), Riikonen *et al.* (177) found that $\alpha_2\beta_1$ induces MMP-1 (interstitial collagenase) expression, and $\alpha_1\beta_1$ attenuates collagen a1(I) gene expression. In monocytes, several monocyte adherence derived (MAD) inflammatory genes have been identified by screening cDNA libraries derived from adherent monocytes (171). Furthermore, antibody cross-linking of β_1-integrins but not β_2-integrins results in the transcription of inflammatory

TABLE 1. Genes induced by extracellular matrix proteins and integrins

ECM substrate or integrin	Regulated gene ↑ (Up-regulated) ↓ (Down-regulated)	Reference
Fibronectin	↑ c-*fos*, ↑ c-*myc*	176
	↑ TNFα, ↑ CSF-1	185
	↑ junB, ↑ ras	17
	↑ MAD-2, ↑ MAD-5, ↑ MAD-6, ↑ MAD-9	171
	↑ NF-κB	199
120-kDa fragment of fibronectin	↑ Gelatinase B, ↑ stromelysin	198
	↑ Collagenase, ↑ c-*fos*, ↑ c-*jun*	180
Collagen	↑ MAD-2, ↑ MAD-5, ↑ MAD-6, ↑ MAD-9	171
Laminin[a]	↑ β-Casein, ↑ β-Lactoglobulin	173, 172
$\alpha_2\beta_1$	↑ Collagenase	177
$\alpha_1\beta_1$	↓ Collagen α1(I)	177
$\alpha_5\beta_1$	↑ gas-1, ↑ Bcl-2	22, 87
β_1	↓ ICE	31
β_1	↑ Tissue factor	183
β_1	↑ IL-1, ↑ IL-1ra, ↑ MAD-6	169
Serum[b]	↑ Cyclin A	191

CSF-1, Colony-stimulating factor 1; ICE, interleukin-1β converting enzyme.

[a] Requires lactogenic hormones.

[b] Vitronectin and fibronectin are constituents of serum. Nonadherent cells do not induce cyclin A; only cells adhered to plastic in the presence of serum will induce cyclin A.

mediator genes (interleukin 1β, interleukin 1 receptor antagonist, and MAD-6) (169). Although the β_2-integrins in monocytes do not induce gene expression, engaging the β_2-integrins in monocytes before β_1-activation suppresses MAD-6 expression (169).

Regulation of gene expression by FN is complex, owing to its many binding sites for cell surface receptors and other matrix molecules. For example, only basal levels of collagenase and gelatinase are expressed when fibroblasts are plated on FN, but when these cells are plated on the 120-kDa RGD-containing fragment of FN, expression of these two genes is enhanced (175). The induction of collagenase and gelatinase by the 120-kDa fragment is mediated by $\alpha_5\beta_1$. Suppression of collagenase and gelatinase expression occurs when the CS-1 fragment of FN (CS-1 fragment does not contain the 120-kDa fragment) stimulates the $\alpha_4\beta_1$-receptor. Thus, intact FN will generate at least two signals emanating from the 120-kDa fragment and the Cs-1 region, with the $\alpha_4\beta_1$-signal dominating. Additionally, *in vivo*, proteinases like collagenase are found in wound fluid at sites of inflammation where FN fragments have also been found, suggesting that these fragments may be similar in activity to the 120-kDa fragment (178). Gene expression induced by FN is also influenced by the presence of tenascin (179). Tenascin is a glycoprotein in the ECM that binds to cell surface proteoglycans and also to FN. When cells are plated on a mixture of tenascin and FN, the cells behave as though they were plated on the 120-kDa fragment of FN; MMPs and c-*fos* levels are increased (179). This effect is specific for FN as tenascin has no effect either alone or in combination with either VN or Col (179). The mechanism responsible for tenascin's effects is unknown.

The transcription factor-binding sites in the promoters of integrin-induced genes have also been examined. Tremble *et al.* (180) have recently demonstrated that the collagenase promoter contains AP1 and PEA3 sites, which are both required for collagenase expression induced by the 120-kDa fragment of FN. AP1 sites bind c-*fos* and c-*jun*, whose expression precedes the induction of collagenase when fibroblasts are plated on the 120-kDa fragment of FN (180). AP-1 sites are also found in promoters of other MMPs like gelatinase B and stromelysin-1, which are induced by the FN 120-kDa fragment (181, 182). Whether the AP-1 sites or other binding sites in the promoters of gelatinase B and stromelysin-1 are required for integrin-induced gene expression is not clear. Two AP-1 sites and a κB-like binding site are part of an integrin-responsive element in the promoter for tissue factor (TF) gene in monocytes (183). The integrin-reponsive element is required for full expression of TF by either α_4- or β_1-integrins (183). Finally, a 160-bp transcriptional enhancer (BCE1) regulates ECM and PRL induction of β-casein expression (184).

Promoters of many genes induced by adherence of monocytes to plastic contain NF-kB binding sites (2, 6, 169, 185). Although plastic does not mimic any specific ECM protein, many genes that are induced by monocyte adherence to plastic have been found to be induced by FN or other ECM proteins (2, 6, 169, 185). Monocytes also induce expression of IkB upon adherence, and the binding of IκB to NF-κB in the cytoplasm is thought to inhibit NF-κB's activity. Thus, expression of IκB induced by integrins may negatively regulate or limit integrin-mediated gene expression by NF-κB (170). Genes down-regulated by monocyte adherence contain c-*myb* and helix-loop-helix binding sequences in their promoters and not NF-kB sites (6).

The signal transduction pathways responsible for inducing gene expression by integrins have not been completely defined. In monocytes, a tyrosine kinase(s) inhibited by genistein or herbomycin appears to regulate expression of interleukin-1β (186). FAK does not play a role in regulating gene expression in these cells since monocytes do not express FAK (169, 186). In epithelial cells, signals generated by cytokines and laminin-1 are required for expression of β-lactoglobulin. The transcription factor Stat5 can only bind to its recognition site in the β-lactoglobulin promoter if both PRL and laminin-1 interact with the cells (172). Both c-*jun* and c-*fos*, which are required for the AP-1-dependent transcription of some integrin-induced genes, can be activated by MAP kinase family members. c-*jun* Is also activated by the Rho family of GTPases while c-*fos* transcription can be induced by Rho, in a MAP kinase-independent manner (162, 180, 187, 188). Integrins may regulate AP-1-dependent transcription through the activation of MAP kinases and/or Rho. The regulation of gene expression *in vivo* probably relies on the integration of signals from both growth factor receptors and integrins. Elucidation of signaling pathways stimulated by both of these receptors will enhance our understanding of how cells regulate gene expression.

An important function of integrin-controlled gene expression may be the regulation of cell growth and cell survival. In addition to c-*fos* and c-*myc* (as discussed above), integrins have also been shown to regulate the expression of other growth-related genes such as Ras, c-*jun*, junB, cyclin A, and gas-1 (22, 176, 189–191). Dike and Farmer (176) found that the expression of c-*fos* and c-*myc* did not depend on the presence of growth factors, suggesting that integrins can regulate the G0/G1 transition. Integrins have also been shown to induce cyclin A expression and regulate cyclin A-dependent kinase activity (189–191). Cyclin A is required for cell cycle progression into S phase (192). Symington (189, 190) found that ligand binding by $\alpha_5\beta_1$ was sufficient to stimulate cyclin A-associated kinase activity. Guadagno *et al.* (191) observed that the expression of cyclin A, but not cyclin D1, cyclin E, cdc2, or cdk2, depended on cell adhesion in NRK cells and NIH3T3 fibroblasts. Moreover, unregulated expression of cyclin A enabled the NRK cells to proliferate in suspension. These results link cyclin A expression to integrin-mediated growth control.

As discussed earlier, overexpression of integrins in tumor cells can suppress anchorage-independent growth in some systems. Varner *et al.* (22) report that this effect is due to the integrin-mediated expression of the growth arrest gene, Gas-1, which is known to block cell cycle progression (193). Gas-1 is a growth arrest-specific gene that functions to block cell cycle progression. In addition to Gas-1 induction, overexpression of $\alpha_5\beta_1$ inhibited the expression of the growth-associated genes c-*fos*, c-*jun*, and junB (22). Based on these findings, it appears that integrin expression alone, in the absence of ligand binding and receptor clustering, is sufficient to modulate gene expression under certain conditions.

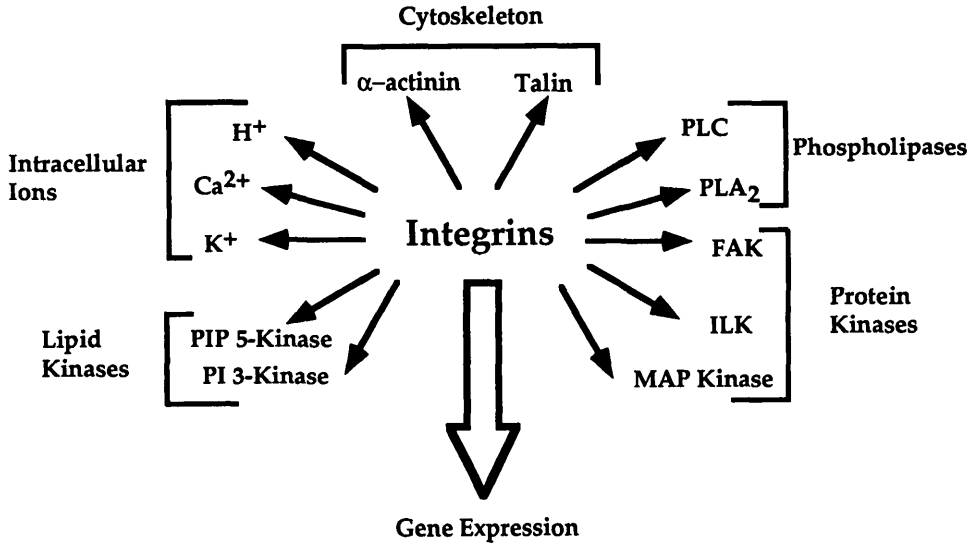

FIG. 3. Integrin-mediated signaling. In addition to their role as adhesion receptors, integrins also induce many signaling events. Integrins have been shown to regulate the levels of the intracellular ions H^+, Ca^{2+}, and K^+, reorganization of the cytoskeleton through interactions with α-actinin and talin, lipid metabolism including lipid hydrolysis via activation of the phospholipases PLC and PLA_2 and lipid synthesis via activation of the lipid kinases PIP 5-kinase and PI 3-kinase, protein phosphorylation through activation of FAK, ILK, and MAP kinase, and finally gene expression. Many of these integrin-mediated signaling events may be interdependent. The regulation of gene expression may require multiple components leading from the plasma membrane to the nucleus.

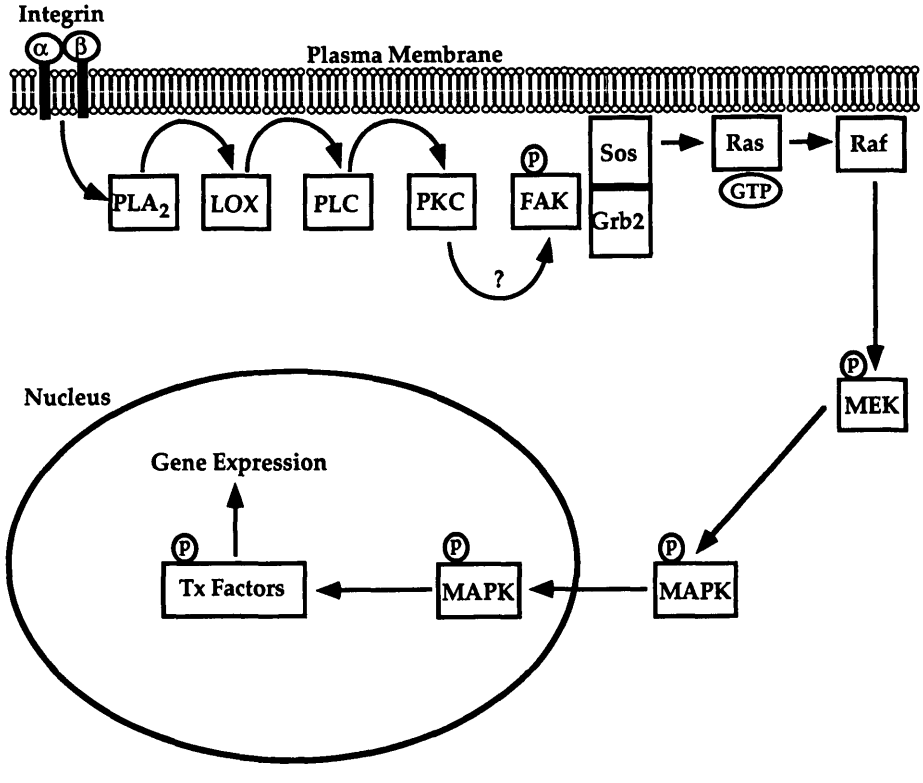

FIG. 4. Proposed signaling pathway for integrin-mediated gene expression. One possible signaling pathway leading from the plasma membrane to the cell nucleus is shown. In this pathway, integrin-mediated activation of the phospholipase PLA_2 leads to PLC activation via production of arachidonic acid and the subsequent generation of a lipoxygenase (LOX) metabolite. Activated PLC then induces an increase in DAG, which in turn stimulates PKC. PKC then activates FAK, although not through direct phosphorylation of FAK. Tyrosine-phosphorylated FAK then binds to the Grb2-Sos complex, which leads to the subsequent activation of Ras. Activated Ras then stimulates Raf kinase, which then phosphorylates and activates MEK, which in turn phosphorylates and activates MAP kinase. Activated MAP kinase then translocates to the nucleus where it phosphorylates and activates different transcription factors.

Integrin-mediated gene expression is also required for the regulation of cell survival. The induction of PCD depends on the balance of inhibitors, such as Bcl-2, and activators, such as interleukin 1β-converting enzyme proteases (194–196). Zhang *et al.* (87) found that ligation of $\alpha_5\beta_1$ in CHO cells induced the expression Bcl-2, which correlated with cell survival. In mammary epithelial cells, disruption of contact with the ECM induced expression of interleukin 1β-converting enzyme and activated PCD in these cells (31). These results indicate that integrins both activate and suppress expression of death-associated genes, depending upon the cell type and the cellular environment.

IV. Conclusion

Integrin-ligand binding and subsequent activation lead to the induction of many diverse signaling events (Fig. 3). In general, these integrin-mediated signaling events can be grouped as either proximal, near the plasma membrane (*e.g.* reorganization of the cytoskeleton), or distal, within the nucleus (*e.g.* changes in gene expression). Although both proximal and distal events have been studied in some detail (as discussed above), the signals that bridge these events are largely unknown. A potential pathway from integrin activation to gene expression might involve PLA_2, PLC, PKC,

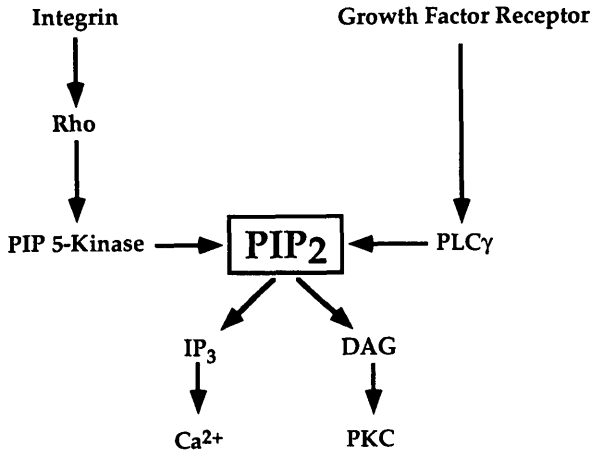

FIG. 5. Synergism between growth factor receptor and integrin receptor pathways. The synergy between integrins and growth factor receptors may depend on the ability of integrins to regulate the amount of substrate available for growth factor receptor-activated pathways. In this example, integrins regulate the levels of the lipid PIP_2 by the Rho-dependent activation of PIP 5-kinase. Activated growth factor receptors, such as the PDGF receptor, phosphorylate and activate PLCγ which then catalyzes the hydrolysis of PIP_2 to IP_3 and DAG. The synthesis of PIP_2 catalyzed by PIP 5-kinase may be the critical rate-limiting step for the IP_3-dependent regulation of intracellular Ca^{2+} and the DAG-dependent activation of PKC.

FAK, components of the Ras/Raf pathway, MAP kinase, and *fos/jun* activation (Fig. 4). Further dissection of these pathways will ultimately provide insight into integrin-mediated growth control and cell survival.

In vivo a cell receives information from both the ECM and growth factors, molecules that activate integrins and growth factor receptors, respectively. Accumulating evidence suggests that these signaling pathways are not independent but interact within the cell. Throughout the preceding text many examples of this "synergy" between integrins and growth factor receptors were discussed. These examples support a general model in which integrins modulate the relative levels of substrate available for growth factor receptor-activated pathways.

One example of this is the ability of integrins to induce the formation of focal adhesions. Focal adhesions contain many growth factor receptor substrates (*e.g. src*, PLCγ, PI 3-kinase, FAK) in addition to some growth factor receptors. Integrin-induced formation of these focal adhesion "signaling complexes" would function to increase the local concentration of these molecules, thereby facilitating their interactions after growth factor stimulation. This might explain the observation that in many cases both integrins and growth factor receptors can activate the same effector molecule (*e.g.* Rho, FAK, PI 3-Kinase), observations that are somewhat disturbing given that normal cells require both types of signals to proliferate. Possibly, formation of these signaling complexes, in the absence of growth factors, may be sufficient to activate some of these effector molecules. One prediction of this model would be that in the absence of integrin signaling, growth factors would still be active but not as effective.

A second mechanism that illustrates the ability of integrins to regulate the relative levels of substrate involved in growth factor-activated pathways is shown in Fig. 5. In this example, integrins regulate the amount of the phospholipid PIP_2 available to the growth factor receptor-activated PLCγ. Hydrolysis of PIP_2, by PLCγ, then activates downstream events that are part of the growth-factor receptor signal transduction pathway. One prediction of this model is that regulation of PIP_2 levels, independent of integrin signaling, would induce anchorage-independent growth. The ability of integrins to regulate the metabolism of other lipids may also be related to this mechanism.

Knowledge of the integrin-mediated regulation of cell growth and cell survival may facilitate our understanding of many key aspects of development and physiology. Indeed, integrin-mediated cell survival appears to play a major role in the coordination of events during organ regression (31) and may be involved in tube morphogenesis during development (197). In addition, integrin-mediated cell proliferation in lymphocytes may regulate inflammatory responses. The elucidation of integrin-mediated signal transduction pathways should provide insights into the regulation of these phenomena.

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