Invited Review

Integrin-mediated signal transduction pathways

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Summary. Integrins serve as adhesion receptors for extracellular matrix proteins and also transduce biochemical signals into the cell. They regulate a variety of cellular functions, including spreading, migration, proliferation and apoptosis. Many signaling pathways downstream of integrins have been identified and characterized and are discussed here. In particular, integrins regulate many protein tyrosine kinases and phosphatases, such as FAK and Src, to coordinate many of the cell processes mentioned above. The regulation of MAP kinases by integrins is important for cell growth or other functions, and the putative roles of Ras and FAK in these pathways are discussed. Phosphatidylinositol lipids and their modifying enzymes, particularly PI 3-kinase, are strongly implicated as mediators of integrin-regulated cytoskeletal changes and cell migration. Similarly, actin cytoskeleton regulation by the Rho family of GTPases is coordinated with integrin signaling to regulate cell spreading and migration, although the exact relationship between these pathways is not clear. Finally, intracellular pH and calcium fluxes by integrins are suggested to affect a variety of cellular proteins and functions.

Key words: Extracellular Matrix (ECM), Focal Adhesion Kinase (FAK), Mitogen-Activated Protein (MAP) Kinase, Phosphatidylinositol 3-kinase (PI 3-kinase)

Introduction

Extracellular matrix (ECM) proteins such as fibronectin, vitronectin, laminin and collagen form a complex network to which cells attach and respond. These cell-ECM interactions are important for a variety of biological processes. ECM proteins are recognized by cell surface receptors, one family of which is the integrins (reviewed in Hynes, 1992). At present at least 16 distinct integrin α subunits and 8 β subunits have been identified which can combine to form at least 20 different receptors. The combination of a particular α and β polypeptide confers the specificity of the receptor for the ECM ligand; for example, the α5β1 integrin is a well characterized fibronectin receptor. Integrins not only serve as adhesion molecules, but can also transduce biochemical signals into the cell to regulate a variety of cellular functions, including cell proliferation, apoptosis and migration (reviewed in Ruoslahti and Reed, 1994; Lauffenburger and Horwitz, 1996; Assoian, 1997; Bottazzi and Assoian, 1997). The purpose of this review is to describe these signaling pathways as a step towards understanding the mechanisms of integrin regulation of cellular functions.

Discussion

Protein tyrosine kinases and phosphatases

Integrin activation results in increased tyrosine phosphorylation of several different cellular proteins (Guan et al., 1991; Kornberg et al., 1991; Burridge et al., 1992), and many of the kinases involved in these pathways have been identified. Perhaps the most extensively studied of these is focal adhesion kinase (FAK), a non-receptor protein tyrosine kinase which is localized to focal adhesions (Hanks et al., 1992; Schaller et al., 1992). FAK demonstrates both increased kinase activity (Guan and Shalloway, 1992; Lipfert et al., 1992) and tyrosine phosphorylation (Guan et al., 1991; Kornberg et al., 1991; Burridge et al., 1992; Hanks et al., 1992; Lipfert et al., 1992) in response to integrin activation, which is dependent on an intact integrin β cytoplasmic tail (Guan et al., 1991). The major site of FAK autophosphorylation in vivo and in vitro has been mapped to Y397 (Chan et al., 1994; Schaller et al., 1994; Eide et al., 1995), which serves as a binding site for the SH2 domains of Src family members (Schaller et al., 1994; Eide et al., 1995) and phosphatidylinositol 3-kinase (PI 3-kinase) (Chen et al., 1996a). FAK has been demonstrated to play a role in several integrin-mediated cellular events, including cell migration (Ilic et al., 1995; Cary et al., 1996; Gilmore and Romer, 1996), cell proliferation (Gilmore and Romer, 1996; Zhao et al., 1998), and cell death or anoikis (Frisch et al., 1996; Hungerford et al., 1996; Xu et al., 1996). FAK-regulated
cell migration is dependent on Y397 (Cary et al., 1996), which allows for both Src-mediated phosphorylation of FAK-associated p130Cas (Cary et al., 1998) and an independent PI 3-kinase-mediated pathway (Reiske et al., 1998). FAK-regulated cell proliferation (Zhao et al., 1998) and anoikis (Frisch et al., 1996) also require Y397, suggesting roles for Src and/or PI 3-kinase in these events as well. Furthermore, FAK-regulated anoikis may involve caspase-mediated cleavage of FAK to generate a FRNK-like polypeptide (Crouch et al., 1996; Wen et al., 1997; Gervais et al., 1998; Levkau et al., 1998). In addition, FAK is suggested to play a role in integrin-mediated cell spreading from studies with the C-terminal FAK-related non-kinase (FRNK) (Richardson and Parsons, 1996; Richardson et al., 1997). Therefore FAK is clearly an important regulator of several integrin-initiated signal transduction pathways.

Another tyrosine kinase regulated by integrins is Src (reviewed in Thomas and Brugge, 1997), which demonstrates increased kinase activity in fibroblasts plated on fibronectin (Kaplan et al., 1995; Schlaepfer et al., 1998). The Src family members Hck and Fgr are also important in β3 integrin-mediated signaling in neutrophils (Berton et al., 1994; Lowell et al., 1996). Src clearly functions intimately with FAK in many integrin signaling pathways, as mentioned above, and it may play additional roles in integrin signaling as well. While c-Src is normally localized with endosomal membranes in fibroblasts (Kaplan et al., 1992), under some conditions various Src constructs are localized to focal adhesions or similar structures (Howell and Cooper, 1994; Kaplan et al., 1994, 1995; Fincham et al., 1996; Fincham and Frame, 1998), as is the negative-regulatory Src kinase Csk (Howell and Cooper, 1994; Bergman et al., 1995). Interestingly, Src−/− cells demonstrate decreased spreading on fibronectin (Kaplan et al., 1995) while Csk overexpression results in decreased cell adhesion (Bergman et al., 1995), suggesting that Src plays a role in these integrin-mediated events.

A third tyrosine kinase with a role in integrin signaling is c-Abl, which contains a protein tyrosine kinase domain, SH2 and SH3 domains, and actin and DNA-binding domains (reviewed in Wang, 1993). Transformation of cells in culture by the chimeric oncogene Bcr/Abl results in anchorage-independent but serum-dependent cell growth (Renshaw et al., 1995). Further evidence that Abl is involved in integrin signaling is the demonstration that Bcr/Abl regulates proteins with known or suggested functions in integrin signaling, including p130Cas and Casp, paxillin, Crk, PI 3-kinase, and tensin (Salgia et al., 1995, 1996; de Jong et al., 1997; Skorski et al., 1997). In addition, c-Abl activity is decreased in detached cells and increased upon fibronectin repletion, and a fraction of activated c-Abl is transported to the nucleus where it may act on specific genes (Lewis et al., 1996). The functional role of c-Abl in integrin signal transduction is not currently known.

The protein tyrosine kinase Syk is regulated by integrins in various leukocytes. Integrin β3 activation in neutrophils results in increased tyrosine phosphorylation of Syk and a correlative increase in interleukin-18 expression through an NF-KB-dependent mechanism (Lin et al., 1994, 1995). Increased activity and phosphorylation of Syk has been demonstrated upon integrin activation in a variety of cell types (Clark et al., 1994; Keely and Parise, 1996; Gao et al., 1997; Yan et al., 1997). The function of Syk in response to integrins is not clear, but it is suggested to regulate gene expression based on the correlation of these events.

Protein tyrosine kinases clearly play an important role in integrin signaling, and dephosphorylation of their substrates by specific phosphatases (reviewed in Denu et al., 1996; Tonks and Neel, 1996) is also an important factor in these pathways. Several specific phosphatases involved in integrin function have been identified. Both association of the phosphatase SHP-2 with its substrate SHPS-1 as well as tyrosine phosphorylation of SHPS-1 are regulated by cell adhesion to ECM (Fujisaka et al., 1996), effects which are dependent on FAK and Src regulation (Tsuda et al., 1998). Regulation of these proteins by SHP-2 is believed to affect integrin-dependent MAP kinase activation (Tsuda et al., 1998) as well as focal adhesion formation and cell migration (Yu et al., 1998). The phosphatase PTEN regulates FAK phosphorylation, focal adhesion formation, cell spreading and migration, effects which are suggested to contribute to its functions as a tumor suppressor gene (Tamura et al., 1998). In addition, several phosphatases interact with the integrin-regulated signaling molecules p130Cas (Garton et al., 1996; Liu et al., 1996; Black and Bliska, 1997) and paxillin (Shen et al., 1998). In particular, PTP1B association with and dephosphorylation of p130Cas is believed to regulate mitogen-activated protein (MAP) kinases, cell spreading and migration by integrins (Iliu et al., 1998). Thus the tyrosine phosphorylation as well as dephosphorylation of many proteins are important mechanisms of integrin signaling.

Mitogen-activated protein kinases

Regulation of several protein serine/threonine kinases by integrins has been well documented. Best characterized of these pathways involve the MAP kinases, particularly the Erk (extracellular signal-regulated kinase) subfamily. Activation of integrins, either by plating cells on ECM proteins or by receptor cross-linking using anti-β1 antibodies, results in activation of Erks in a number of cell types (Chen et al., 1994; Morino et al., 1995; Zhu and Assoian, 1995). In addition to Erks, activation of the JNK (Jun N-terminal kinase) subfamily of MAP kinases also occurs by fibronectin (Miyamoto et al., 1995).

Based on the well established role of Ras in regulating MAP kinases by mitogenic stimuli, an important question is whether integrins also regulate MAP kinases through Ras. At present the answer to this
question is not clear. The involvement of Ras is supported by the early observations of its activation by integrins (Kapron-Bras et al., 1993; Clark and Hynes, 1996; Zheng et al., 1996). Integrin-mediated Erk activation is blocked by a dominant-negative Ras construct in several cell types (Clark and Hynes, 1996; Schlaepfer and Hunter, 1997; Wei et al., 1998). However, in another system, fibronectin does not activate Ras, nor does a dominant-negative Ras inhibit fibronectin-stimulated Erk activation (Chen et al., 1996). Thus while the role for the Raf kinase is well-supported (Lin et al., 1997b), the role of Ras is not clear. A recent study may provide an explanation for these results, as it demonstrates a time-dependent response of Ras, such that Ras is required only for an initial phase of Raf and Erk activation by integrins but not for a sustained response (Howe and Juliano, 1998).

FAK has been suggested to play a role in MAP kinase activation by integrins, but like that of Ras, the role of FAK in this pathway is controversial. It was first suggested that FAK may mediate MAP kinase activation by its association with Grb2 at FAK Y925 (Schlaepfer et al., 1994), a hypothesis supported by the demonstration that transient FAK expression in HEK 293 cells enhances fibronectin-stimulated Erk activation (Schlaepfer and Hunter, 1997; Schlaepfer et al., 1998). However, several studies have demonstrated a dissociation of FAK tyrosine phosphorylation and MAP kinase activation (Seufferlein et al., 1996; Lin et al., 1997a); and furthermore, FAK overexpression in a Chinese hamster ovary (CHO) cell system does not result in the activation of MAP kinases (Caray et al., 1998). The explanation for these conflicting results is not clear. They may reflect cell-specific signaling pathways. It is also possible that complete FAK phosphorylation is not necessary for its promotion of Erk activity, therefore a dissociation of these events does not indicate a functional dissociation of these proteins. Clearly, future work is needed to resolve these discrepancies.

In systems where a clear role for FAK in fibronectin-stimulated Erk activation has been demonstrated, this pathway has been extensively studied. It was originally suggested that Src binding to Y397 of FAK allows for Src phosphorylation of FAK Y925, Grb2 binding, and subsequent activation of Erks (Schlaepfer et al., 1994; Schlaepfer and Hunter, 1996). However, while Y925 is only partially required for Erk activation by FAK, Y397 is completely necessary (Schlaepfer and Hunter, 1997; Schlaepfer et al., 1998). Three different pathways have therefore been suggested to mediate Erk activation by the FAK/Src complex: a major pathway involving tyrosine phosphorylation of She and its association with Grb2 (Schlaepfer et al., 1998), a minor pathway involving FAK/Grb2 association, and another minor pathway involving phosphorylation of FAK-associated p130Cas (Schlaepfer et al., 1997). Further supporting the importance of She in integrin regulation of MAP kinases, a dominant-negative She construct inhibits integrin-mediated Erk activation, although this is likely to occur through a FAK/Src-independent pathway (Wary et al., 1996). Thus there are believed to be several pathways downstream of integrins in the regulation of MAP kinases.

The functional role of integrin-stimulated Erk activation may be several-fold. Primarily, it is believed to act synergistically with mitogenic signaling pathways to regulate cell growth (Lin et al., 1997b; Eliceiri et al., 1998; Short et al., 1998). In G0-synchronized NIH 3T3 cells, for example, PDGF is responsible for a rapid and transient MAP kinase activation, while fibronectin mediates a slower, sustained response, and both stimuli are required for maximal DNA synthesis (Zhu and Asoolian, 1995). In addition, Erk activation has been suggested to regulate integrin-dependent cell spreading and migration (Klemke et al., 1997; Reszka et al., 1997). Finally, while R-Ras affects integrin affinity to increase cell adhesion on fibronectin (Zhang et al., 1996), H-Ras suppresses integrin functions, an effect which correlates with Erk activation (Hughes et al., 1997). These results suggest that Erks may act in a feedback mechanism downstream of Ras to regulate integrin receptors. Clearly the MAP kinases are important mediators of integrin signaling, and an understanding of the mechanisms as well as the outcome of their involvement in these pathways is central to an understanding of integrin signaling.

Phosphatidylinositol lipids

Phosphatidylinositol lipids have been identified as second messengers in a variety of signal transduction pathways. In addition to serving as a substrate for phospholipase Cγ, PI(4,5)P2 has also been shown to regulate actin cytoskeleton dynamics by directly binding to a number of cytoskeletal proteins. These include profilin (Lassing and Lindberg, 1985), gelsolin (Janmey et al., 1987), capping protein (Schafer et al., 1996), α-actinin (Fukami et al., 1992), vinculin (Gilmore and Burridge, 1996), and ezrin/radixin/moesin proteins (Hirao et al., 1996). These effects of PI(4,5)P2 are believed to increase F-actin polymerization, bundling, and linking to the plasma membrane. Indeed, reduction in the amount of PI(4,5)P2 by expression of a specific phosphatidylinositol phosphatase decreases actin stress fibers and bundling (Sakisaka et al., 1997).

Like other signaling pathways, integrins have also been shown to regulate various phosphatidylinositol-modifying enzymes. Integrin stimulation of mouse fibroblasts by plating on fibronectin results in increased levels of PI(4,5)P2 (McNamee et al., 1993), which is possibly a result of Rho-mediated activation of PI(4)P 5-kinase (Chong et al., 1994; Ren et al., 1996). This suggests an attractive model in which integrin-mediated generation of PI(4,5)P2 affects actin cytoskeleton by the mechanisms described above. Activation of integrin α2β3 in platelets results in increased levels of PI(3)P, which may be produced by either the inositol 5-phosphatase SHIP (Giuriato et al., 1997). PI(3)P 4-
kinase (Banfic et al., 1998), and/or PI 3-kinase (Guinebault et al., 1995). This effect coincides with translocation of PI 3-kinase to the actin cytoskeleton (Guinebault et al., 1995). In addition, platelet spreading on fibrinogen is dependent on PI 3-kinase (Heraud et al., 1998). PI 3-kinase involvement in integrin signaling has been shown in other cell types as well. Cell migration on collagen is mediated by PI 3-kinase, which is predicted to act downstream of Rac and/or Cdc42 (Keely et al., 1997). Carcinoma cell invasion by the α5β1 integrin is dependent on its activation of PI 3-kinase (Shaw et al., 1997). Finally, an adhesion-dependent association of PI 3-kinase with FAK has been shown (Chen and Guan, 1994) which is mediated by direct binding of PI 3-kinase to FAK Y397 (Chen et al., 1996a) and which regulates CHO cell migration on fibronectin (Reiske et al., 1998). Integrins therefore regulate phosphatidylinositolides through various modifying enzymes, resulting in effects on actin cytoskeleton dynamics, cell spreading and cell migration.

**Rho family GTPases**

Regulation of the actin cytoskeleton and subsequent cellular functions by the Rho family of Ras-related low molecular weight GTPases has been well characterized (reviewed in Hall, 1994; Tapon and Hall, 1997). Cdc42 controls the formation of filopodial extensions, Rac controls lamellipodia and membrane ruffling through regulation of cortical actin, and Rho controls actin stress fibers and formation of focal adhesions. These events are all important aspects of cell adhesion, spreading and motility and are also events mediated by integrins, but the relationship between integrin signaling and Rho family members is still not clear.

Many studies have suggested that Rho lies upstream of integrin signaling pathways and possibly integrins themselves. Cell transformation by the Rho exchange factors Dbl or Lbc induces anchorage-independent cell growth (Schwartz et al., 1996). Fibronectin matrix assembly, an integrin-dependent event, is also regulated by Rho (Zhang et al., 1994) and likely occurs through its effects on the actin cytoskeleton (Zhang et al., 1997). More specifically, some studies have shown that focal adhesion formation is dependent on Rho and Rac (Hotchin and Hall, 1995) and that complete formation of actin stress fibers by Rho is dependent on integrins (Machesky and Hall, 1997), suggesting that integrin signaling does not occur without first being activated from within the cell by these GTPases. Further supporting this hypothesis are several studies demonstrating that Rho lies upstream of FAK. Treatment of cells with lypo-phosphatidic acid (LPA) to activate Rho results in increased FAK kinase activity (Rodriguez-Fernandez and Rozengurt, 1998) as well as tyrosine phosphorylation (Barry and Critchley, 1994; Ridley and Hall, 1994; Seufferlein and Rozengurt, 1994), and this effect is inhibited by specific ADP-ribosylation and inhibition of Rho (Kumagai et al., 1993). Furthermore, the formation of stress fibers by activated Rho requires a tyrosine kinase (Ridley and Hall, 1994), and since FAK phosphorylation is increased by activated Rho (Flinn and Ridley, 1996) it is proposed to be one such kinase.

On the other hand, some studies have suggested that integrins are upstream of these GTPases (Bourouleaux et al., 1998). Integrin activation by either fibronectin or anti-β3 antibodies activates PAK, a downstream effector of Rac and Cdc42 (Price et al., 1998). In addition, integrin aggregation induces the colocalization of p190-B, a protein with GAP activity for Rho. Rac and Cdc42 (Burbelo et al., 1995), and also induces its tyrosine phosphorylation (Nakahara et al., 1996). A resolution to these seemingly conflicting conclusions may be that this pathway is not linear but rather is cyclical. Cell contractility is required for the formation of stress fibers and focal adhesions by Rho (Chrzanowska-Wodnicka and Burridge, 1996). Recently, Clark et al. (1998) demonstrated that early focal adhesion formation and regulation of FAK/Src in cells on ECM is mediated independently of Rho proteins, but that subsequently Rho GTPases regulate the complete formation of focal adhesion and stress fibers. These results suggest that integrins may initiate focal adhesion formation and signal transduction pathways. Upon activation of Rho proteins, perhaps in an integrin-dependent manner, stress fibers are formed and contracted, which in turn ultimately affects integrins and integrin signaling. Thus integrins may be both dependent and independent of Rho family GTPases; clearly further studies are needed to reconcile these seemingly conflicting observations.

**Intracellular ion fluxes**

Regulation of intracellular pH by integrins was first suggested by Schwartz et al. (1989), who demonstrated that the degree of cell spreading correlated with intracellular pH levels. This is an effect of the α5β1 integrin, independent of cell shape, and regulated by the Na+/H+ antiporter (Schwartz et al., 1991). Similarly, plating endothelial cells on increasing concentrations of fibronectin results in increased intracellular pH, which is controlled by the Na+/H+ antiporter (Inger et al., 1990). As with other integrin-mediated events, regulation of intracellular pH occurs synergistically with growth factors in some cell types (Inger et al., 1990; Schwartz and Lechene, 1992). Furthermore, integrin-dependent regulation of intracellular pH can occur by the Rho GTPase, and is believed to be necessary for Rho-mediated effects on the actin cytoskeleton, focal adhesions, cell adhesion and cell spreading (Tominaga and Barber, 1998).

Integrins also regulate cytosolic calcium levels. Activation of integrins in various cell types results in increased cytosolic Ca2+ (Pardi et al., 1989; Ng-Sikorski et al., 1991; Leavesley et al., 1993; Schwartz, 1993). These calcium transients are believed to be mediated by both influx of extracellular calcium as well as release of intracellular calcium stores (Schwartz, 1993; Sjaastad et
al., 1996). These calcium fluxes may activate a positive feedback loop to activate integrins (Sjaastad et al., 1994), and they may also be important for efficient cell migration (Marks et al., 1991; Hendey and Maxfield, 1993; Leavesley et al., 1993). Based on its distribution within a migrating cell (lowest at the leading edge and highest at the cell rear) (Hahn et al., 1992), calcium is believed to regulate proteins responsible for release of the cell rear. Indeed, the calcium-dependent protease calpain is required for efficient migration mediated by either β1 or β3 integrins (Huttenlocher et al., 1997), which may be due to its cleavage of such focal adhesion proteins as talin and Src (Schoenwaelder et al., 1997). In addition, inhibition of calcineurin, a Ca2+/calmodulin-dependent protein phosphatase, inhibits neutrophil migration on vitronectin, apparently as a result of inefficient release of the cell rear (Hendey et al., 1992).

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