Journal of Cellular Biochemistry 61:543–553 (1996)

Integrin-Dependent Signal Transduction

Robert M. Lafrenie and Kenneth M. Yamada

Laboratory of Developmental Biology, National Institute of Dental Research, National Institutes of Health, Bethesda, Maryland 20892-4370

Abstract Integrins are receptor molecules for extracellular matrix molecules (e.g., the β_1 family), serum components (α_v family) and immunoglobulin family adhesion molecules (β_2 family). Integrin-dependent adhesion has also been shown to have metabolic consequences. Adhesion to a variety of extracellular matrix proteins, such as fibronectin, collagen, and laminin, is a potent regulator of cell growth, differentiation, and gene expression. Ligand binding or aggregation of integrin receptors initiates a number of metabolic changes including activation of serine/threonine and tyrosine kinases, increased Ca^{2+} influx, increased cytoplasmic alkalinization, and altered inositol lipid metabolism. In some instances activation of transcription factors and induction of gene expression have also been demonstrated. Components of key signaling pathways involving integrins are beginning to be identified. Some studies have shown that integrins form multi-component complexes with signal transduction molecules. Elucidating the interactions of the signal transduction molecules with each other and with the integrin cytoplasmic domains will be key to understanding the initial events of signal transduction through the integrins. © 1996 Wiley-Liss, Inc.*

Integrins are a large superfamily of cell surface receptors composed of non-covalent heterodimers of an α and a β subunit. More than 20 different members of the integrin family can be generated in vertebrates by pairing at least 15 different α chains with at least 8 different β chains [Hynes, 1992]. These molecules are involved in a number of cell-cell and cell-matrix interactions, which are important in several biological phenomena, including development, thrombosis, wound healing, and cancer. Until recently, the only described role for integrins was that of mediating adhesion. For example, the β_1 family integrins mediate cell adhesion to extracellular matrix molecules and to VCAM-1. The α_v family of integrin adhesion molecules are more promiscuous, mediating cell adhesion to many extracellular matrix proteins and to serum components. The β_2 integrins, expressed on leukocytes, bind to immunoglobulin family adhesion molecules expressed on activated endothelial cells, thus mediating cell/cell adhesion. However, integrin-dependent adhesion also alters normal cell function and activates various complex signal transduction pathways that change cell behavior [for current reviews see Juliano and Haskell, 1993; Sastry and Horwitz, 1993; Hemler et al., 1994; Schwartz, 1994]. Thus, adhesion to a variety of extracellular matrix proteins, such as fibronectin, collagen, and laminin, can regulate cell growth, differentiation, and gene expression.

It is currently not possible to construct a comprehensive model of signal transduction arising from integrin binding to ligands, but several general principles seem to recur (Fig. 1). First among these is the apparent requirement for receptor clustering. Adhesion of cells to extracellular matrix-coated surfaces results in receptor clustering into adhesion plaques and along fibrils of matrix proteins. In many experimental systems, interactions with integrins by monovalent ligands, such as Fab' antibody fragments or small soluble ligands, are unable to initiate downstream effects unless these ligands are subsequently dimerized or clustered [Werb et al., 1989; Kornberg et al., 1991; Clark et al., 1994a]. Thus, it has been speculated that ligand binding induces receptor clustering which then triggers another event, such as initiation of a protein phosphorylation cascade, which leads to secondary changes. It is thought that an immediate consequence of receptor clustering is the formation of complexes in which the cytoplasmic domains of the cell surface receptor interact with various cytoplasmic proteins and organize

Received May 2, 1995; accepted December 7, 1995.

Address reprint requests to Robert M. Lafrenie, NIDR/DIR/LDB, Bldg. 30, Rm. 408, 30 Convent Dr. MSC-4370, Bethesda, MD 20892-4370.

Fig 1. **Steps involved in integrin-dependent signal transduction**

1. Ligand binding
2. Receptor clustering
3. Intracellular events
   i. assembly of focal contacts and organization of the cytoskeleton
   ii. activation of serine/threonine and tyrosine kinases
      protein phosphorylation of:
         -ECM receptors (β_1 integrins?)
         -focal contact proteins (FAK, paxillin, tensin, talin)
         -src-family kinases (*Src, Csk, Syk Nck, Fyn*)
   iii. increased $Ca^{2+}{}_{IN}$
   iv. increased pH_{IN}
   v. altered inositol metabolism
4. Activation of transcription factors
5. Transcription of specific genes

changes in the cytoskeleton [Burridge et al., 1988].

A number of intracellular events that are presumed to be mediators of signal transduction have been described. For example, integrin-mediated adhesion increases the phosphorylation of a large number of proteins including extracellular matrix receptors, focal contact and cytoskeletal proteins, and *Src*-family kinase members [Hemler et al., 1994; Schwartz, 1994]. Further, activation of protein kinase C (PKC) has been demonstrated. Alterations in intracellular Ca^{2+} and intracellular pH as well as changes in inositol lipid metabolism also result from integrin occupation and clustering. Since this spectrum of changes in cellular metabolism is similar to the changes seen in response to growth factor stimulation [Hill and Treisman, 1995], it is not surprising that the signal transduction pathways involved in growth factor responsiveness have also been implicated in adhesion-dependent signal transduction. However, the pathways which relay signals from sites of cell/matrix contact to the nucleus, where changes in gene transcription eventually result from integrin occupation, are largely unknown.

RELATIONSHIP BETWEEN ADHESION AND GROWTH FACTOR RESPONSIVENESS

Cell-matrix and cell-cell adhesion are capable of modifying cellular responses to growth factors. For example, the ability of FGF to stimulate proliferation of an embryonic carcinoma cell line is highly dependent on adhesion to fibronectin or laminin [Schubert and Kimura, 1991]. A murine mammary carcinoma cell line proliferates in response to PDGF or bFGF when plated on fibronectin but not collagen [Elliot et al., 1992]. Since plating cells on these matrices did not change the number or affinity of growth factor receptors, it appears that integrins differ in their ability to provide conditions that permit growth factor signaling. These systems seem quite complex. In some cases integrin occupation/activation seems to be of secondary importance or to act merely as a permissive signal. In many cases, integrin occupation and growth factor/agonist treatment both appear to be required, and they act synergistically. Thus, models which describe cellular perturbation in response to integrin-dependent adhesion may also involve costimulation of growth factor-dependent signal transduction pathways.

Insulin treated cells that are cultured on vitronectin show much more DNA synthesis than when attached to other substrates. This increase correlates with the association of insulin receptor substrate-1 (which associates with the GRB2 signaling complex) and the $\alpha_v\beta_3$ integrin in insulin-treated cells [Vuori and Ruoslahti, 1994]. This physical association suggests a mechanism for the synergistic action of growth factor and integrin receptors.

INTEGRIN-DEPENDENT CHANGES IN GENE EXPRESSION

Non-Adherent Vs. Adherent Cells

The simplest experiments to analyze the effects of integrin-dependent adhesion on cellular activation are those in which cells are plated onto integrin ligands. For example, monocytes, normally grown in suspension, upregulate expression of a number of immediate-early genes, including IL-1β, PDGF, *c-fos, c-jun,* and EGR2 when they adhere to fibronectin or collagen, but also to plastic substrates [Shaw et al., 1990; Lin et al., 1994]. Expression of some of these genes is also induced within 1 h by antibody-dependent crosslinking of the β_1 integrin. Upregulated gene expression is inhibited by inclusion of tyrosine phosphorylation inhibitors, suggesting a role for tyrosine kinases in integrin signal transduction [Lin et al., 1994]. A somewhat more complicated example of gene induction is found in the ability of CD4+ (T) cells to respond synergistically to CD3- and integrin-mediated signals for induction of IL-2 mRNA and enhanced cell proliferation on fibronectin substrates [Yamada et al., 1991]. CD3-stimulation induces activation of the NFAT-1 transcription factor, while $\alpha_5\beta_1$ occupation independently activates the AP-1 transcription factor (via a PKC-independent pathway), and activation of both factors is required for IL-2 gene induction.

It has also been shown that fibroblasts maintained in suspension express low levels of growth-

related genes, but when plated on fibronectin show rapid increases in *fos* (5 min) and *myc* (30 min) expression, independent of soluble mitogens or cell growth [Dike and Farmer, 1988]. It has been subsequently shown that expression of α1(I)-collagen is also upregulated further downstream [Dhawan and Farmer, 1990]. This increase in collagen mRNA is the result of both increased transcription rate and increased mRNA stability, suggesting there are alterations in both pre- and post-transcriptional events in response to adhesion [Dhawan et al., 1991]. These results suggest that signaling rapidly follows cell contact with the extracellular matrix.

Cell adhesion is intimately involved in regulating cell growth. Normal fibroblasts maintained in suspension are unable to divide, but upregulate synthesis of cyclin A mRNA and pass through the late G1 phase of the cell cycle when adherent [Guadagno et al., 1993]. Treatment of FA-K562 cells with the GRGDS peptide, which interacts with $\alpha_5\beta_1$, increases their anchorage-independent growth and correlates with increased cyclin A-associated kinase and retinoblastoma protein phosphorylation [Symington, 1992]. Further, transfection of NRK cells with cyclin A cDNA promoted multiple rounds of cell division independent of cell adhesion [Guadagno et al., 1993]. Thus cyclin A is a possible target of the adhesion-dependent signals that control cell proliferation.

Transcription of Specific Genes by Cells in Monolayer Culture

The transcription of a variety of different genes has been shown to be upregulated by adherent cells in response to integrin-mediated signaling. Treatment of rabbit synovial fibroblasts with fibronectin fragments or with anti-$\alpha_5\beta_1$ monoclonal antibodies has been reported to increase transcription of collagenase (MMP-1) and stromelysin (MMP-3) [Werb et al., 1989]. Synovial fibroblasts pretreated with soluble anti-$\alpha_5\beta_1$ antibodies (which probably bind near the ligand-binding site) for only 2 h was sufficient to induce collagenase and stromelysin expression after 24 h. In subsequent studies, collagenase induction was seen in as little as 4 h [Tremble et al., 1992]. This response required $\alpha_5\beta_1$ aggregation, since Fab' fragments were not active unless clustered by a secondary antibody, but was independent of the substratum because induction was seen on fibronectin-, vitronectin-, or collagen-coated substrates [Werb et al., 1989]. Curiously, culturing cells on fibronectin surfaces, which involves $\alpha_5\beta_1$, did not induce collagenase expression although culture on anti-$\alpha_5\beta_1$ antibody- or fibronectin fragment (containing the cell-binding domain)-coated surfaces did. Induction was also seen when the fibronectin fragments were presented in a soluble form. It has been suggested that since cells in suspension express high levels of collagenase, harvesting the cells for the experiment enhances collagenase expression, and that the alternately spliced CS-1 domain, present in intact fibronectin but not in the fibronectin fragments, initiates signals which inhibit collagenase expression [Huhtala et al., 1995]. Possible effects of cell shape were excluded by showing that altering the substratum and the inducing agent could result in increases in gene expression independently of cell shape changes [Werb et al., 1989].

Melanoma cells have also been shown to upregulate metalloproteinase production in response to antibody-mediated ligation of integrin receptors. Treatment of the human melanoma cell line A375M with different anti-$\alpha_v\beta_3$ antibodies (including antibodies with no affect on adhesion), but not with anti-$\alpha_5\beta_1$ antibodies, caused an increase in the synthesis and secretion of the 72 kDa metalloproteinase (MMP-2) [Seftor et al., 1992]. However, the highly invasive C8161 melanoma cell line, which expresses very little $\alpha_v\beta_3$, was shown to upregulate 72 kDa metalloproteinase production in response to treatment with anti-$\alpha_5\beta_1$ antibodies [Seftor et al., 1993]. In addition, human keratinocytes upregulate the expression of the 92 kDa metalloproteinase (MMP-9) after treatment with anti-β_1 antibodies [Larjava et al., 1993]. Thus, it appears that aggregation of different integrin receptors is capable of inducing metalloproteinase expression, which is in some way dependent on the integrin repertoire of the cell.

Culturing keratinocytes on collagen gels (Vitrogen) was associated with increased synthesis and release of interstitial collagenase (MMP-1) compared to cells grown on plastic, gelatin, or Matrigel (which contains type IV collagen) [Sudbeck et al., 1993]. Treatment of cells on collagen gels with either tyrosine phosphorylation inhibitors or with PKC inhibitors abolished collagenase upregulation. PKA, PKG, G-protein, or PGE synthesis inhibitors had no effect, suggesting that this response was dependent on both

tyrosine phosphorylation- and PKC-mediated signal transduction pathways.

MATRIX-DEPENDENT DIFFERENTIATION MODELS

The composition of the extracellular matrix and its interaction with the cells of many developing tissues has been shown to strongly influence the differentiation of cells in those tissues. Although a wide variety of tissue culture models which demonstrate the involvement of extracellular matrix in cell differentiation have been described, two models are particularly instructive in understanding adhesion-dependent signal transduction: the mammary epithelial cell and hepatocyte differentiation models.

Hepatocyte Differentiation Model

Isolation of hepatocytes from their normal environment and their culture on plastic or collagen, in the presence of growth factors, results in dedifferentiation, characterized by a flattened cell shape, an increase in cell proliferation, and loss of most liver-specific markers [Dipersio et al., 1991]. However, when these cells are cultured for 2–3 days on extracellular matrix gels, such as Vitrogen (collagen I) or Matrigel (basement membrane extract), they assume their differentiated morphology and express high levels of liver-specific genes, including albumin. Overlaying cells cultured on collagen with a thin layer of Matrigel upregulate albumin production without detectable changes in cell shape suggesting that such changes are not a primary regulator of gene expression [Caron, 1990]. A conditionally transformed murine hepatocyte cell line, H2.35, showed an increase in albumin gene expression when cultured in a hormonally defined serum-free medium on a collagen gel matrix or Matrigel [Dipersio et al., 1991]. Under these conditions activation of three transcription factors, eE-TF, eG-TF/HNF3, and eH-TF, are required to upregulate albumin gene transcription [Liu et al., 1991]. The eE-TF and eH-TF DNA-binding activities increased only when cells were cultured on collagen gels in the hormonally defined medium, but activation of eG-TF/HNF3 only required culturing cells on collagen gels. Thus it appears that the interaction of hepatocytes with collagen gels initiates a signal transduction pathway resulting in at least two separate signals: one which acts in combination with growth factor-dependent signals and a second which acts alone to activate transcription factor binding.

Mammary Epithelial Differentiation Model

Extracellular matrix also strongly influences mammary epithelial cell differentiation in culture. When cultured on extracellular matrix gels such as Matrigel or Vitrogen for 3–7 days in the presence of prolactin, mouse mammary epithelial cells form spheroids reminiscent of in vivo alveoli and express various milk proteins (e.g., caseins, lactoferrin, and transferrin) [Streuli et al., 1991]. As with the hepatocyte model, both hormone and extracellular matrix signals are required, and these signals may act through different promoter elements [Schmidhauser et al., 1992]. Epithelial cells imbedded as a single-cell suspension in laminin-rich Matrigel were able to synthesize β-casein [Streuli et al., 1991]. In contrast, cells suspended in a collagen I matrix did not, unless accompanied by cell-cell contact. Within the matrix, signals appeared to be mediated through integrin receptors, since inclusion of an anti-β_1 integrin antibody inhibited β-casein production. Thus, since laminin and collagen I can utilize different integrin receptors, the difference in β-casein production could be related to associations with different signal transduction mechanisms (although a contribution from growth factors in the Matrigel cannot be ruled out). Using a gel overlay system, it was shown that β-casein production evolved over 3–5 days in cells that became rounded and clustered and was associated with β_1 integrin aggregation and elevated tyrosine phosphorylation of a number of proteins [Roskelley et al., 1994].

SIGNAL TRANSDUCTION PATHWAYS INITIATED AT INTEGRINS

Signalling Via Platelet $\alpha_{IIb}\beta_3$

Much work related to integrin-stimulated signal transduction has been conducted on platelets [reviewed in Shattil et al., 1994]. Since the platelet lacks a nucleus and therefore transcriptional regulation, its ability to respond to environmental cues is dependent on intracellular signal transduction mechanisms. Platelet $\alpha_{IIb}\beta_3$ usually exists as a low-affinity receptor. Treatment of the platelet with soluble agonists, such as thrombin or ADP, initiates an intracellular G-protein-dependent pathway (inside-out signaling) which dramatically increases the affinity of $\alpha_{IIb}\beta_3$ for fibrinogen [Clark et al., 1994b]. Vari-

ous lines of evidence, including the finding that some monoclonal antibodies are able to activate $\alpha_{IIb}\beta_3$ binding in the absence of soluble ligands, and that ligand binding changes $\alpha_{IIb}\beta_3$ susceptibility to proteases, suggests that this activation is based on changes in conformation [Huang et al., 1993]. Fibrinogen binding may then cause oligomerization of $\alpha_{IIb}\beta_3$, and thereby promote interactions between the β_3 cytoplasmic domain and cytoskeletal proteins such as α-actinin and talin, resulting in reorganization of the cytoskeleton. These changes are also associated with the clustering and activation of various protein tyrosine kinases leading to a first wave of tyrosine phosphorylation of proteins including *Syk* and a 140 kDa protein [Clark et al., 1994b]. Aggregation of activated and stirred platelets leads to additional reorganization of the cytoskeleton and probably recruitment of additional tyrosine kinases into a focal contact-like structure which includes vinculin, α-actinin, talin, spectrin, phosphoinositol 3-kinase, GAP, and PKC [Shattil et al., 1994]. Several protein tyrosine kinases such as *Src, Yes,* and FAK also associate with these clusters and are activated in a second round of tyrosine phosphorylation [Lipfert et al., 1992]. Since early tyrosine phosphorylation precedes aggregation and subsequent phosphorylation of *Src* and FAK, it has been suggested that the composition of the cytoskeleton dictates a hierarchy of signaling events during platelet activation [Shattil et al., 1994]. This hypothesis is supported by the observation that fibrinogen binding, in the absence of agonist stimulation, leads to the phosphorylation of *Syk* but is not sufficient for *Src* or FAK phosphorylation [Huang et al., 1993]. Phosphorylation of FAK requires additional, coordinated activation signals from both the integrin-dependent and the agonist-activated PKC and Ca^{2+} influx pathways [Shattil et al., 1994].

AGGREGATION OF SIGNALING COMPLEXES AND TYROSINE PHOSPHORYLATION

As discussed for several of the above models, signal transduction mechanisms originating from integrin receptors involve a number of different signal transduction pathways, including tyrosine kinase and PKC pathways. Since the cytoplasmic domains of integrins do not possess enzymatic activity, the ability of integrins to initiate signals must depend on recruited signal transduction molecules. Thus, molecules that accumulate with integrins under conditions sufficient for signal transduction probably include the molecules that enzymatically translate the signal. Areas of integrin clustering on the cell surface where cell-substratum adhesion occurs often involve structures termed focal adhesions or focal contacts which are very complex collections of molecules [reviewed in Burridge et al., 1988]. The focal contact appears to play an important structural role in the maintenance of strong cell/substrate adhesions and in the promotion of cell spreading, and thus interactions between the integrin cytoplasmic domains at these sites and a large number of cytoskeletal components are not surprising (Fig. 2). At least two of these cytoskeletal proteins, talin [Tapley et al., 1989] and α-actinin [Otey et al., 1990] appear to be capable of interacting directly with the β_1 cytoplasmic domain. Talin and α-actinin bind zyxin, paxillin, and vinculin, which in turn, bind tensin [Burridge et al., 1988]. Tensin and α-actinin bind actin filaments, thus linking the cell surface integrin to the actin cytoskeleton. However, it is important to emphasize that there are several types of cell adhesive contacts with extracellular matrix molecules, and that the cytoskeletal associations in each still require more characterization. For example, cells can adhere to even simple substrates such as fibronectin by means of at least three types of contact: focal contacts, matrix contacts (also termed ECM contacts), and close contacts [Chen and Singer, 1982]. These contacts all involve integrin interactions with fibronectin, although the matrix contacts involve the binding of integrins to fibronectin fibrils. The types of cytoskeletal molecules involved and the sequence in which they bind to integrin cytoplasmic domains in each type of contact are still obscure. For example, even though tensin is sometimes depicted at the end of the chain of interactions ending in actin filaments, it may also initiate the chain, since tensin is the only cytoskeletal protein that accumulates when β_1 integrin is aggregated, in the absence of ligand occupation [Miyamoto et al., 1995].

Molecules involved in mediating signal transduction must also have their activities changed in response to integrin-dependent signal initiation. Integrin-mediated cell adhesion to extracellular matrix components or aggregation of integrin receptors by specific anti-integrin antibodies has been shown to induce phosphorylation on tyrosine residues of a number of proteins [Kornberg et al., 1991]. In particular, one of these

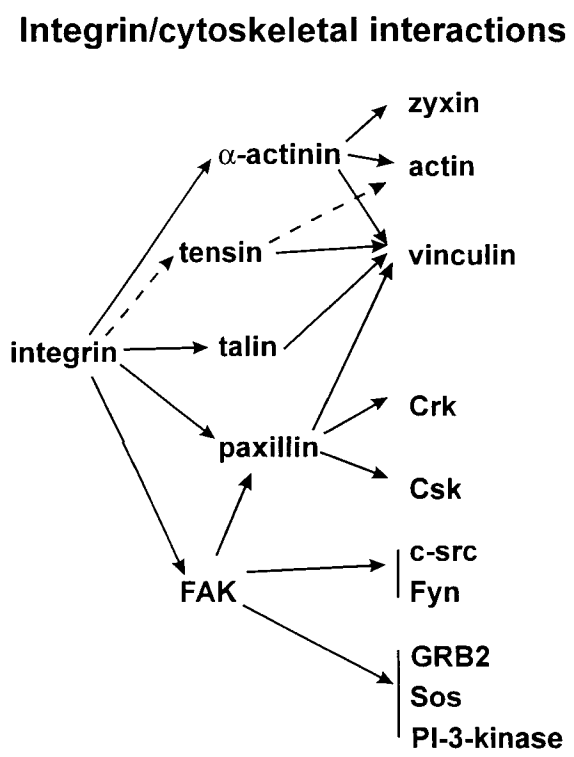

Fig. 2. Interactions between various cytoplasmic components of the focal contact as determined from in vitro binding data. *Arrows* indicate direct interactions proposed between the various components.

proteins, focal adhesion kinase (FAK or $pp125^{FAK}$), is phosphorylated rapidly and localizes to focal adhesions [Guan et al., 1991; Kornberg et al., 1992]. The mechanism by which integrin activation induces FAK phosphorylation is unknown, but evidence supporting direct binding of FAK to the β_1 cytoplasmic domain [Hildebrand et al., 1993] suggests that changes in integrin aggregation and/or conformation alters FAK or its environment promoting autophosphorylation [Parsons et al., 1994]. Simply clustering integrins with non-inhibitory antibodies, in the absence of ligand-occupation (and thus presumably without ligand-induced conformational changes) is sufficient to induce FAK phosphorylation [Miyamoto et al., 1995]. Thus, simply increasing the local concentration of FAK, and associated molecules, can promote autophosphorylation. This is apparently different from the situation in platelets where FAK phosphorylation also requires PKC and Ca^{2+}-mediated signals [Shattil et al., 1994]. Several other cytoplasmic proteins found in focal adhesions, such as paxillin, talin, vinculin, and tensin, are also tyrosine phosphorylated, although specific information concerning the enzymes that mediate these phosphorylations are currently unknown [Burridge et al., 1992].

The separate roles of integrin-ligand binding and integrin aggregation have been examined by measuring the accumulation of various cytoskeletal and cytoplasmic proteins at sites of cell contact with beads coated with anti-integrin antibodies or fibronectin [Miyamoto et al., 1995]. Aggregation of $\alpha_5\beta_1$ in the absence of ligand occupation, induced by beads coated with a non-inhibitory anti-integrin antibody, results in co-clustering of $\alpha_5\beta_1$ with FAK and tensin. Under these conditions, FAK autophosphorylation is concomitant with clustering. When integrins are similarly clustered in the presence of soluble ligand, or with antifunctional integrin antibodies, $\alpha_5\beta_1$ coaggregates with phosphorylated FAK, tensin, vinculin, paxillin, α-actinin, talin, and actin. This result suggests that $\alpha_5\beta_1$ can exist on the cell surface in association with FAK and tensin (or that association is rapidly induced) and that ligand occupation alters integrin conformation to permit accumulation of additional cytoskeletal components. Since α-actinin and talin can bind directly to the β_1 integrin cytoplasmic domain, ligand occupation must expose their binding sites. However, this accumulation of cytoskeletal components does not follow from FAK phosphorylation, since FAK autophosphorylation can occur without triggering cytoskeletal accumulation.

If these events originate from integrin signaling, then sequences in the β_1 cytoplasmic domain should be required for cytoskeletal reorganization and changes in FAK phosphorylation. Experiments utilizing a chimeric receptor composed of the cytoplasmic domain of the β_1 integrin and the extracellular domain of a non-signaling IL-2 receptor subunit show that aggregation of isolated β_1 cytoplasmic domains is sufficient to induce phosphorylation of FAK [Akiyama et al., 1994]. The β_1 cytoplasmic domain has been shown to contain all of the information needed to target the receptor to focal adhesions, suggesting that it mimics the conformation of ligand-occupied integrin and its ability to bind cytoplasmic components [LaFlamme et al., 1994]. Mutational studies have also shown that the sequences which localize β_1 to focal contacts are required for FAK phosphorylation [Guan et al., 1991]. Some experimental models suggest that phosphorylation of integrin subunits is involved in focal contact interactions and signal transduction. In particular, cell transformation by *v-Src* causes increased tyrosine

phosphorylation of a small proportion of β_1 cytoplasmic domains, along with the phosphorylation of several cytoskeletal components, causing disorganization of the cytoskeleton, a relocalization of phosphorylation β_1, and a decrease in cell/substratum adhesion [Johansson et al., 1994]. However, conflicting evidence also exists [reviewed in Hemler et al., 1994], and general principles regarding integrin phosphorylation and signal transduction are inconclusive at present.

Much attention has focused on the SH2 and SH3 domains found in many phosphorylated focal contact proteins [Cantley and Songyang, 1994; Cohen et al., 1995]. These domains mediate the molecule-molecule interactions found among many components of the focal contact, and their affinities are modulated by phosphorylation. For example, phosphorylated paxillin is able to bind to several potential signalling molecules, such as the phosphotyrosine kinase *Src* through its SH3 domain [Weng et al., 1993], and to *Csk* (a kinase that phosphorylates and negatively regulates *Src*) through its SH2 domain [Sabe et al., 1994]. GRB2, an SH2-containing adapter protein, has been shown to bind to both phosphorylated FAK and to *c-Src,* resulting in the activation of MAP kinase [Schlaepfer et al., 1994]. In *v-Src* transfected cells, phosphorylated FAK binds to the *Src* SH2 domain [Xing et al., 1994] and the FAK/*Src*/GRB2/*Sos* signaling complex exists independent of cell adhesion [Schlaepfer et al., 1994]. These results suggest that FAK autophosphorylation and paxillin phosphorylation in response to integrin-mediated signals initiate binding to other intracellular signaling molecules that mediate interactions between integrins and *Ras*/MAP kinase signal transduction pathways. The role of cytoskeletal assembly is unclear, since conditions where FAK is phosphorylated in the absence of cytoskeletal accumulation have been described [Miyamoto et al., 1995]. Nevertheless, disruption of actin integrity by cytochlasin D inhibits FAK phosphorylation [Parsons et al., 1994]. The apparent ability of cells to form functional signaling complexes in the absence of major cytoskeletal involvement suggests that integrins have two general cytoplasmic functions: (1) cytoskeletal organization, which is important in various structural responses to adhesion and in migration, and (2) tyrosine kinase signal transduction in response to extracellular matrix signals; these two functions are experimentally separable (Fig. 3).

PROTEIN KINASE C

PKC type 3 has been localized to focal adhesions in some cell types and is associated with changes in actin microfilament organization [Jaken et al., 1989]. This suggests a possible involvement in signal transduction. Various components of focal contacts are known to be substrates for PKC [Burridge et al., 1988]. Further, as previously discussed, PKC inhibitors block the secretion of collagenase by keratinocytes cultured on type I collagen gels [Sudbeck et al., 1993]. In a number of instances where extracellular matrix proteins regulate cellular function, exogenous activation of PKC can replace the requirement for adhesion. For example, extension of neurites on low concentrations of laminin is inhibited by PKC inhibitors and can be induced to the same level as seen on high concentrations of laminin by PKC activators [Bixby and Jhabvala, 1990]. Some normal fibroblasts attach to and spread on the cell-binding domain of fibronectin, but do not assemble focal contacts efficiently unless they interact with the heparin-binding domain of fibronectin. This requirement for the heparin-binding domain can be overcome by treatment with phorbol esters, suggesting that a second signal is mediated via PKC [Woods and Couchman, 1992]. PKC is also required for HeLa cell spreading. In this system, adhesion of HeLa cells to collagen or gelatin induces rapid release of arachidonic acid, production of diacylglycerol, and translocation of PKC to the plasma membrane before cell spreading can occur [Chun and Jacobson, 1993]. Inhibition of arachidonic acid metabolism or release, or of diacylglycerol synthesis can inhibit cell spreading; this inhibition can also be overcome by phorbol ester treatment.

G-PROTEIN SIGNALING

Integrin-dependent adhesion also appears to modulate G-protein-dependent signal transduction events, since adhesion is shown to alter the actin cytoskeleton, and the actin skeleton is regulated by small GTP-binding proteins, such as *Rac* and *Rho* [reviewed in Hall, 1994]. Once activated, *Rho* and *Rac* promote the formation of actin assembly nucleation sites at the plasma membrane. Although the mechanisms by which *Rho* controls the assembly of functional actin- and integrin-containing complexes is unknown, it seems to promote numerous molecular interactions. It has also been shown that a G protein $\tau 5$ subunit is found in focal adhesions of many

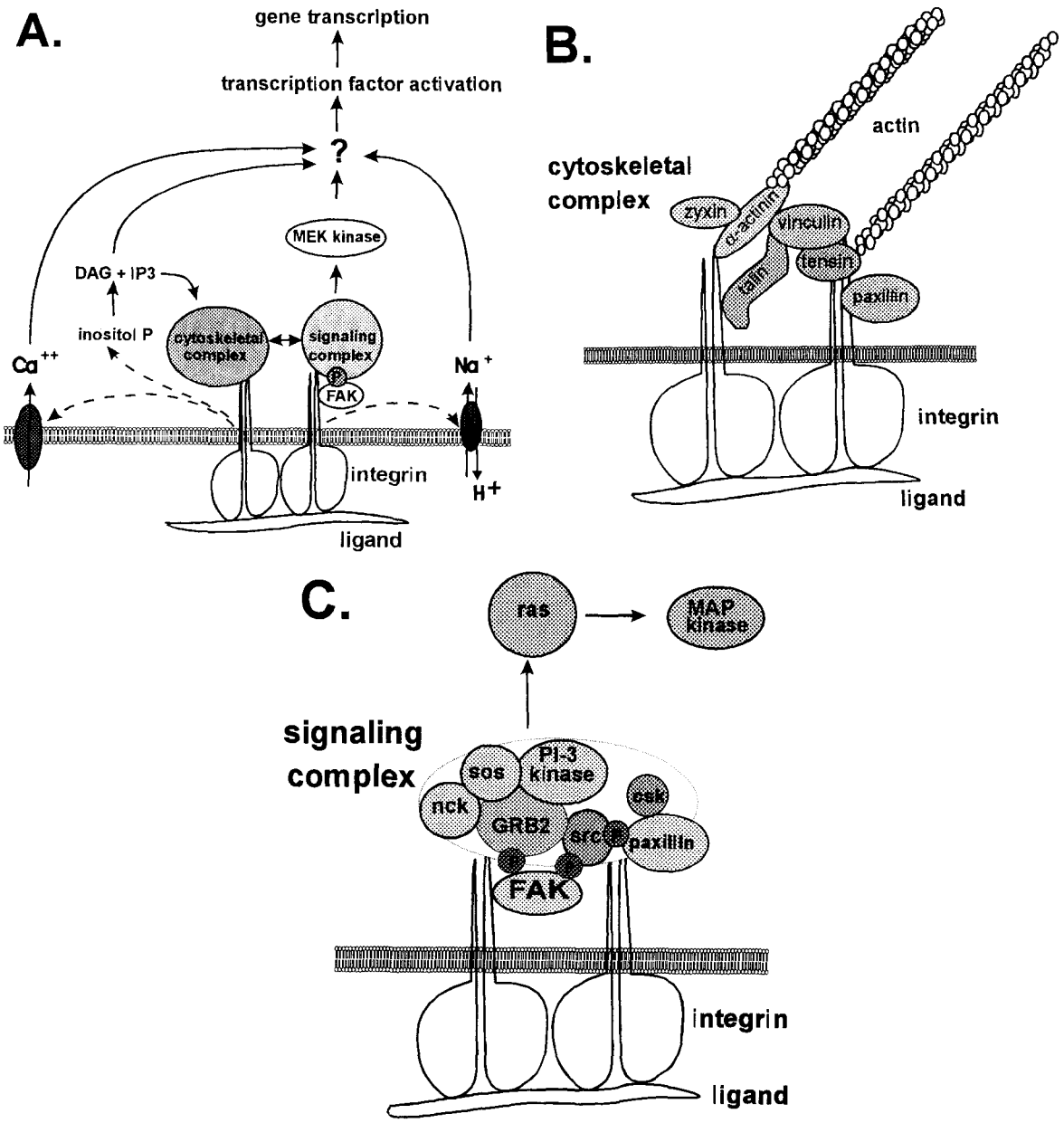

Fig. 3. Integrin-dependent signal transduction pathways. This model shows a number of different signal transduction pathways proposed to result from integrin binding to ligand and subsequent aggregation ending in upregulated gene transcription (**A**). The model emphasizes a divergence in integrin interactions with a cytoskeletal complex (**B**) and with a signaling complex (**C**).

different cell types [Hansen et al., 1994]. Antibody staining is extracted with Triton X-100, suggesting that it localizes to the plasma membrane, and staining completely overlaps with vinculin and to some extent with actin. The localization of a G protein subunit to focal adhesions supports the idea that focal adhesions may be a site where G-protein and extracellular matrix protein-dependent signalling pathways converge to regulate cell adhesion and growth.

There is also a complex interrelationship between the tyrosine kinase signalling pathways and G-proteins. The tyrosine kinase-dependent SH2/SH3 signaling complex includes *Sos*, a *Ras guanine nucleotide exchange factor*, which leads to activation of the MAP kinase signal transduction pathway [Hall, 1994]. In addition, G protein-coupled receptors, including *Rho* which are activated by bombesin treatment, can rapidly induce the tyrosine phosphorylation of several proteins

including FAK [Sinnett-Smith et al., 1993; Seckl et al., 1995], suggesting a potential involvement for G-proteins in tyrosine phosphorylation signals.

INOSITOL LIPID METABOLISM

Adhesion of cells to the extracellular matrix has been correlated with an increase in the levels of intracellular water-soluble inositol phosphates, possibly in response to phospholipase C activation [Cybulsky et al., 1990]. Inositol lipid metabolism is impaired in suspended cells; specifically, inositol bis phosphate (PIP_2) levels are much lower, and adhesion of cells to fibronectin rapidly induces synthesis and accumulation of PIP_2 [McNamee et al., 1993]. PDGF treatment of these adherent cells, but not suspended cells, induces release of phosphoinositides from PIP_2 hydrolysis. PIP_2 hydrolysis results in IP_3 and diacylglycerol release, which in turn results in release of Ca^{2+} from intracellular stores and PKC activation. This system may be another example of a signal transduction pathway where growth factor and integrin-mediated signals interact. Integrins prime the system by increasing PIP_2 levels and growth factors trigger the system by inducing hydrolysis of the PIP_2 to active metabolites.

ACTIVATION OF ION CHANNELS

Cell adhesion and spreading that is mediated by integrins can result in alterations in intracellular Ca^{2+}, and sometimes in oscillations in intracellular Ca^{2+} concentration [Schwartz et al., 1993a]. In endothelial cells, triggering of the $\alpha_v\beta_3$ integrin generates a rise in intracellular Ca^{2+} while triggering through $\alpha_2\beta_1$ did not [Schwartz, 1993]. Monoclonal antibodies against a 50 kDa integrin-associated protein (IAP) block this calcium influx [Schwartz et al., 1993]. In addition, migration of endothelial cells on vitronectin but not collagen was dependent on the presence of extracellular calcium [Schwartz, 1993]. This requirement for calcium may be to activate the phosphatase calcineurin, which dephosphorylates a number of proteins, which then leads to deadhesion [Hendey et al., 1992]. Thus, integrin-dependent changes in intracellular Ca^{2+} seem to be specific for a particular integrin ($\alpha_v\beta_3$) in some cells.

Integrin-dependent cell spreading or antibody-induced clustering of integrins activates the Na^+/H^+ antiporter, producing an elevation of intracellular pH [Schwartz et al., 1991]. However, unlike the changes in Ca^{2+}, all of the integrins tested were capable of altering intracellular pH. These effects are dependent on integrin clustering and immobilization but appear to be independent of changes in cell shape. It has been suggested that this increase in pH could be involved in regulation of cellular growth, but induction of pH_i by fibronectin-coated beads did not induce DNA synthesis. Thus, this system seems to lack the specificity expected of a bona fide signalling pathway. However, changes in pH induced by integrin "activation" is required as one of a number of different signals to stimulate a cellular response.

CONCLUSIONS

Integrin-dependent signal transduction is very complex. A wide variety of transduction pathways have been implicated. However, since many of these pathways appear to interact, the critical steps have not been clearly identified. To further complicate matters, different integrins may somehow induce different signaling pathways, and signaling through the same integrin expressed in different cells may be different. FAK activation appears to be a critical event in integrin signaling by tyrosine phosphorylation, but monocytes, which also signal by tyrosine phosphorylation, do not contain FAK [Shaw et al., 1990]. Much of the current work focuses on signal transduction pathways identified in growth factor and cytokine-activated pathways. However, the ability of integrin-mediated downstream events to synergize with cytokine and growth factor signals suggests at least some divergence in these pathways.

A general model for integrin-dependent signaling can be abstracted from these data. First, ligand occupation and aggregation of integrins result in the phosphorylation of FAK which could then help to recruit a signal transduction complex via SH2/SH3 domain interactions. The signaling complex includes both tyrosine kinase and G-protein signaling molecules similar to the molecules activated in response to growth factor treatment. Integrin occupation also results in enhanced PIP_2 levels, increased cytoplasmic Ca^{2+}, and cytoplasmic alkalinization. Together these, and perhaps other signals, activate transcription factors that promote transcription of specific genes. Challenges for the future include tracing the steps for a complete signaling pathway, from integrin ligation to gene induction, as well as sorting out the interactions between signal transduction pathways. In addition, it will be interesting to learn how different cell and

tissue types modify these signaling pathways to mediate specific cellular functions in cell adhesion, migration, and gene expression in cell growth and differentiation.

REFERENCES

Akiyama SK, Yamada SS, Yamada KM, LaFlamme SE (1994): Transmembrane signal transduction by integrin cytoplasmic domains expressed in single-subunit chimeras. J Biol Chem 269:15961–15964.

Bixby JL, Jhabvala P (1990): Extracellular matrix molecules and cell adhesion molecules induce neurites through different mechanisms. J Cell Biol 111:2725–2735.

Burridge K, Fath K, Kelly T, Nuckolls G, Turner C (1988): Focal adhesions: Transmembrane junctions between the extracellular matrix and the cytoskeleton. Annu Rev Cell Biol 4:487–525.

Burridge K, Turner CE, Romer LH (1992): Tyrosine phosphorylation of paxillin and pp125^{FAK} accompanies cell adhesion to extracellular matrix: A role in cytoskeletal assembly. J Cell Biol 119:893–903.

Cantley LC, Songyang Z (1994): Specificity in recognition of phosphopeptides by src-homology 2 domains. J Cell Sci Suppl 18:121–126.

Caron JM (1990): Induction of albumin gene transcription in hepatocytes by extracellular matrix proteins. Mol Cell Biol 10:1239–1243.

Chen WT, Singer SJ (1982): Immunoelectron microscopic studies of the sites of cell-substratum and cell-cell contacts in cultured fibroblasts. J Cell Biol 95:205–222.

Chun JS, Jacobson BS (1993): Requirement for diacylglycerol and protein kinase C in HeLa cell-substratum adhesion and their feedback amplification of arachidonic acid production for optimum cell spreading. Mol Biol Cell 4:271–279.

Clark EA, Trikha M, Markland FS, Brugge JS (1994a): Structurally distinct disintegrins contortrostatin and multisquamatin differentially regulate platelet tyrosine phosphorylation. J Biol Chem 269:21940–21943.

Clark EA, Shattil SJ, Ginsberg MH, Bolen J, Brugge JS (1994b): Regulation of the protein tyrosine kinase pp72^{syk} by platelet agonists and the integrin $\alpha_{IIb}\beta_3$. J Biol Chem 269:28859–28864.

Cohen GB, Ren R, Baltimore D (1995): Modular binding domains in signal transduction proteins. Cell 80:237–248.

Cybulsky AV, Bonventre JV, Quigg RJ, Wolfe LS, Salant DJ (1990): Extracellular matrix regulates proliferation and phospholipid turnover in glomerular epithelial cells. Am J Physiol 259:F326.

Dhawan J, Farmer SR (1990): Regulation of α1(I)-collagen gene expression in response to cell adhesion in Swiss 3T3 fibroblasts. J Biol Chem 265:9015–9021.

Dhawan J, Lichtler AC, Rowe DW, Farmer SR (1991): Cell adhesion regulates pro-α1(I) collagen mRNA stability and transcription in mouse fibroblasts. J Biol Chem 266:8470–8475.

Dike LE, Farmer SR (1988): Cell adhesion induces expression of growth-associated genes in suspension-arrested fibroblasts. Proc Natl Acad Sci USA 85:6792–6797.

Dipersio CM, Jackson DA, Zaret KS (1991): The extracellular matrix coordinately modulates liver transcription factors and hepatocyte morphology. Mol Cell Biol 11:4405–4414.

Elliot B, Ostman A, Westermark B, Rubin K (1992): Modulation of growth factor responsiveness of murine mammary carcinoma cells by cell matrix interactions: Correlation of cell proliferation and spreading. J Cell Physiol 152:292–301.

Guadagno TM, Ohtsubo M, Roberts JM, Assoian RK (1993): A link between cyclin A expression and adhesion-dependent cell cycle progression. Science 262:1572–1575.

Guan J-L, Trevithick JE, Hynes RO (1991): Fibronectin/integrin interaction induces tyrosine phosphorylation of a 120-kDa protein. Cell Regul 2:951–964.

Hall A (1994): Small GTP-binding proteins and the regulation of the actin cytoskeleton. Annu Rev Cell Biol 10:31–54.

Hansen CA, Schroering AG, Carey DJ, Robishaw JD (1994): Localization of a heterodimeric G protein gamma subunit to focal adhesions and stress fibers. J Cell Biol 126:811–819.

Hemler ME, Weitzman JB, Pasqualini R, Kawaguchi S, Kassner PD, Berdichevsky FB (1994): Structure, biochemical properties, and biological functions of integrin cytoplasmic domains. In Takada Y (ed): "Integrins." Boca Raton, FL: CRC Press, pp 2–35.

Hendey B, Klee CB, Maxfield FR (1992): Inhibition of neutrophil chemokinesis on vitronectin by inhibitors of calcineurin. Science 258:296–299.

Hildebrand JD, Schaller MD, Parsons JT (1993): Identification of sequences required for the efficient localization of the focal adhesion kinase, pp125^{FAK}, to cellular focal adhesions. J Cell Biol 123:993–1005.

Hill CS, Treisman R (1995): Transcriptional regulation by extracellular signals: Mechanisms and specificity. Cell 80:199–211.

Huang M-M, Lipfert L, Cunningham M, Brugge JS, Ginsberg MH, Shattil SJ (1993): Adhesive ligand binding to integrin $\alpha_{IIb}\beta_3$ stimulates tyrosine phosphorylation of novel protein substrates before phosphorylation of pp125^{FAK}. J Cell Biol 122:473–483.

Huhtala P, Humphries MJ, McCarthy JB, Tremble PM, Werb Z, Damsky CH (1995): Cooperative signaling by $\alpha_5\beta_1$ and $\alpha_4\beta_1$ integrins regulates metalloproteinase gene expression in fibroblasts adhering to fibronectin. J Cell Biol 129:867-879.

Hynes RO (1992): Integrins: Versatility, modulation and signaling in cell adhesion. Cell 69:11–25.

Jaken S, Leach K, Klauk T (1989): Association of type 3 proteins kinase C with focal contacts in rat embryo fibroblasts. J Cell Biol 109:697–707.

Johansson MW, Larsson E, Luning B, Pasquale EB, Ruoslahti E (1994): Altered localization and cytoplasmic domain-binding properties of tyrosine-phosphorylated β1 integrin. J Cell Biol 126:1299–1309.

Juliano RL, Haskell S (1993): Signal transduction from the extracellular matrix. J Cell Biol 120:577–585.

Kornberg LJ, Earp HS, Turner CE, Prockop C, Juliano RL (1991): Signal transduction by integrins: Increased protein tyrosine phosphorylation caused by clusterin of β1 integrins. Proc Natl Acad Sci USA 88:8392–8396.

Kornberg L, Earp HS, Parsons JT, Schaller M, Juliano RL (1992): Cell adhesion or integrin clustering increases phosphorylation of a focal adhesion-associated tyrosine kinase. J Biol Chem 267:23439–23442.

LaFlamme SE, Thomas LA, Yamada SS, Yamada KM (1994): Single subunit chimeric integrins as mimics and inhibitors of endogenous integrin functions in receptor localization, cell spreading and migration, and matrix assembly. J Cell Biol 126:1287–1298.

Larjava H, Lyons JG, Salo T, Makela M, Koivisto L, Birkedal-Hansen H, Akiyama SK, Yamada KM, Heino J (1993): Anti-integrin antibodies induce type IV collagenase expression in keratinocytes. J Cell Physiol 157:190–200.

Lin TH, Yurochko A, Kornberg L, Morris J, Walker JJ, Haskill S, Juliano RL (1994): The role of protein tyrosine phosphorylation in integrin-mediated gene induction in monocytes. J Cell Biol 126:1585–1593.

Lipfert L, Haimovich B, Schaller MD, Cobb BS, Parsons JT, Brugge JS (1992): Integrin-dependent phosphorylation and activation of the proteins tyrosine kinase pp125^{FAK} in platelets. J Cell Biol 119:905–912.

Liu J-K, Dipersio CM, Zaret KS (1991): Extracellular signals that regulated liver transcription factors during hepatic differentiation in vitro. Mol Cell Biol 11:773–784.

McNamee HP, Ingber DE, Schwartz MA (1993): Adhesion to fibronectin stimulates inositol lipid synthesis and enhances PDGF-induced inositol lipid breakdown. J Cell Biol 121:673–678.

Miyamoto S, Akiyama SK, Yamada KM (1995): Synergistic roles for receptor occupancy and aggregation in integrin transmembrane function. Science 267:883–885.

Otey, CA, Pavalko FM, Burridge K (1990): An interaction between α-actinin and the β_1 integrin subunit in vitro. J Cell Biol 111:721–729.

Parsons JT, Schaller MD, Hildebrand J, Leu T-H, Richardson A, Otey C. (1994): Focal adhesion kinase: Structure and signalling. J Cell Sci Suppl 18:109–113.

Roskelley CD, Desprez PY, Bissell MJ (1994): Extracellular matrix-dependent tissue-specific gene expression in mammary epithelial cells required both physical and biochemical signal transduction. Proc Natl Acad Sci USA 91:12378–12382.

Sabe H, Hata A, Okada M, Nakagawa H, Hanafusa H (1994): Analysis of the binding of the src homology 2 domain of csk to tyrosine-phosphorylated proteins in the suppression and mitotic activation of c-src. Proc Natl Acad Sci USA 91:3984–3988.

Sastry SK, Horwitz AF (1993): Integrin cytoplasmic domains: Mediators of cytoskeletal linkages and extra- and intracellular initiated transmembrane signaling. Curr Opin Cell Biol 5:819–831.

Schlaepfer DD, Hanks SK, Hunter T, van der Geer P (1994): Integrin-mediated signal transduction linked to Ras pathway by GRB2 binding to focal adhesion kinase. Nature 372:786–791.

Schmidhauser C, Casperson GF, Myers CA, Sanzo KT, Bolten S, Bissell MJ (1992): A novel transcriptional enhancer is involved in the prolactin- and extracellular matrix-dependent regulation of β-casein gene expression. Mol Biol Cell 3:699–709.

Schwartz MA (1993): Spreading of human endothelial cells on fibronectin or vitronectin triggers elevation of intracellular free calcium. J Cell Biol 120:1003–1010.

Schwartz MA (1994): Integrins as signal transducing receptors. In Takada Y (ed): "Integrins." Boca Raton, FL: CRC Press, pp 133–145.

Schwartz MA, Ingber DE, Lawrence M, Springer TA, Lechene C (1991): Multiple integrins share the ability to induce elevation of intracellular pH. Exp Cell Res 195:533–535.

Schwartz MA, Brown EJ, Fazeli B (1993): A 50 kDa integrin-associated protein is required for integrin-regulated calcium entry in endothelial cells. J Biol Chem 268:19931–19934.

Schubert D, Kimura H (1991): Substratum-growth factor collaborations are required for the mitogenic activities on activin and FGF on embryonal carcinoma cells. J Cell Biol 114:841–846.

Seckl MJ, Morii N, Narumiya S, Rozengurt E (1995): Guanosine 5′-3-O-(thio)triphosphate stimulates tyrosine phosphorylation of p125^{FAK} and paxillin in permeabilized Swiss 3T3 cells. J Biol Chem 270:6984–6990.

Seftor REB, Seftor EA, Gehlsen KR, Stetler-Stevenson WG, Brown PD, Ruoslahti E, Hendrix MJC (1992): Role of the αvβ3 integrin in human melanoma cell invasion. Proc Natl Acad Sci USA 89:1557–1561.

Seftor REB, Seftor EA, Stetler-Stevenson WG, Hendrix MJC (1993): The 72 kDa type IV collagenase is modulated via differential expression of αvβ3 and α5β1 integrins during human melanoma cell invasion. Cancer Res 53: 3411–3415.

Shattil SJ, Ginsberg MH, Brugge JS (1994): Adhesive signals in platelets. Curr Opin Cell Biol 6:695–704.

Shaw RJ, Doherty DE, Ritter AG, Benedict SH, Clark RAF (1990): Adherence-dependent increase in human monocyte PDGF(B) mRNA is associated with increases in c-fos, c-jun, and EGR2 mRNA. J Cell Biol 111:2139–2148.

Sinnett-Smith J, Zackary I, Valverde AM, Rozengurt E (1993): Bombesin stimulation of p125 focal adhesion kinase tyrosine phosphorylation. J Biol Chem 268:14261–14268.

Streuli CH, Bailey N, Bissell MJ (1991): Control of mammary epithelial differentiation. J Cell Biol 115:1383–1395.

Sudbeck BD, Parks WC, Welgus HG, Pentland AP (1993): Collagen-stimulated induction of keratinocyte collagenase is mediated via tyrosine kinase and protein kinase C activities. J Biol Chem 269:30022–30029.

Symington BE (1992): Fibronectin receptor modulates cyclin-dependent kinase activity. J Biol Chem 267:25744–25747.

Tapley P, Horwitz A, Buck C, Duggan K, Rohrschneider L (1989): Integrins isolated from Rous sarcoma virus-transformed chicken embryo fibroblasts. Oncogene 4:325–333.

Tremble PM, Damsky CH, Werb Z (1992): Fibronectin fragments, but not intact fibronectin, signalling through the fibronectin receptor induce metalloproteinase gene expression in fibroblasts. Matrix Suppl 1:212–214.

Vuori K, Ruoslahti E (1994): Association of insulin receptor substrate-1 with integrins. Science 266:1576–1578.

Weng Z, Taylor JA, Turner CE, Brugge JS, Seidel-Dugan C (1993): Detection of src homology 3-binding proteins, including paxillin, in normal and v-src-transformed Balb/c 3T3 cells. J Biol Chem 268:14956–14963.

Werb Z, Tremble PM, Behrendtsen O, Crowley E, Damsky CH (1989): Signal transduction through the fibronectin receptor induces collagenase and stromelysin gene expression. J Cell Biol 109:877–889.

Woods A, Couchman JR (1992): Protein kinase C involvement in focal adhesion formation. J Cell Sci 101:277–290.

Xing Z, Chen H-C, Nowlen JK, Taylor SJ, Shalloway D, Guan J-L (1994): Direct interaction of v-src with the focal adhesion kinase mediated by the src SH2 domain. Mol Biol Cell 5:413–421.

Yamada A, Nikaido T, Nojima Y, Schlossman SF, Morimoto C (1991): Activation of human CD4 T lymphocytes. J Immunol 146:53–56.