Sensing the environment: a historical perspective on integrin signal transduction

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focus on
signalling and adhesion

Cell adhesion mediated by integrin receptors has a critical function in organizing cells in tissues and in guiding haematopoietic cells to their sites of action. However, integrin adhesion receptors have broader functions in regulating cell behaviour through their ability to transduce bi-directional signals into and out of the cell and to engage in reciprocal interactions with other cellular receptors. This historical perspective traces the key findings that have led to our current understanding of these important functions of integrins.

Integrins are a large family of receptors that mediate the adhesive interactions of cells. The majority of integrins bind to extracellular matrix (ECM) proteins. However, certain integrins interact with counter-receptors on other cells, soluble plasma proteins, or microorganisms[1]. These heterodimeric receptors, consisting of one of 18 α and one of eight β subunits, were originally referred to as integrins, to denote their function in linking the ECM and the cytoskeleton[2]. However, these receptors can regulate many aspects of cell behaviour other than the cytoskeleton. Signalling enzymes and adaptor proteins regulated by integrin engagement control cell survival, proliferation, motility and differentiation through intimate interactions with the cytoskeleton and with other receptors. During the last 15 years, investigators in this area have identified cellular components that link with integrins to transduce signals. They have also provided some insights into the mechanisms that mediate the coordinate control of cellular processes by integrins and other receptors (see the time line of significant findings in Supplementary Information, Fig. S1).

Early studies on the regulation of cell behaviour by adhesion

Most early studies of cell adhesion focused on characterizing the nature of extracellular adhesive interactions. However, investigations of tumour cells in the mid-60's provided the first hint that adhesion is important in regulating cell behaviour. Normal cells were found to be unable to proliferate when cultured in suspension, and were referred to as 'anchorage-dependent'. In contrast, tumour cells were shown to replicate without attachment to a substratum, and thus escaped anchorage dependence[3,4]. Later, the shape of adherent cells was also
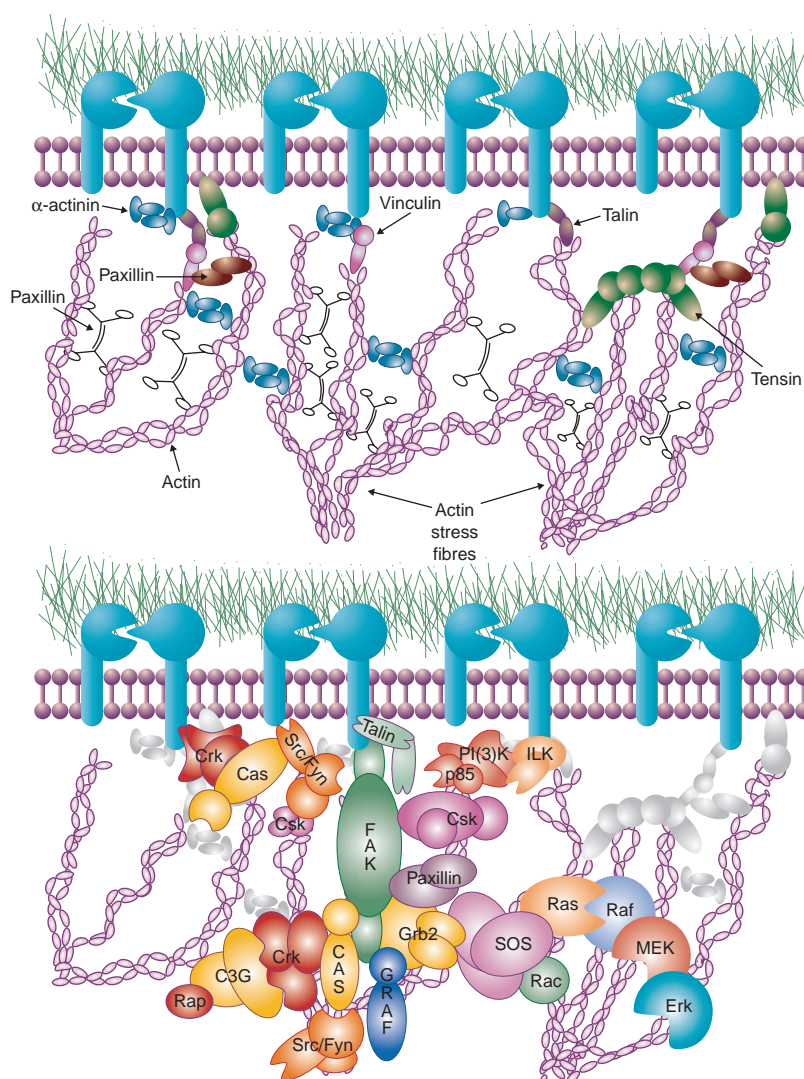

Figure 1 **Cytoskeletal and signalling complexes in focal adhesions. a, Proteins first identified in association with integrins (see text). b, Signal transduction proteins associated with, or activated by, integrins. Signalling molecules, such as FAK, bind to and recruit additional signalling molecules, creating a complex signalling network that is intimately connected to the cytoskeleton network.**

found to be a critical determinant of cell proliferation[5], suggesting that shape-sensitive sensors have an important function in regulating cell proliferation. It was not until two decades later that it was feasible to begin to understand how adhesion and cell shape control this important cellular event.

Adhesion receptors link with the cytoskeleton

Although it was predicted that stable cell adhesion may involve receptor coupling to the cytoskeleton[6], the first evidence that ECM protein receptors are linked to the cytoskeleton came from the observation that the addition of fibronectin (then known as LETS protein or CSP) to tumour cells induced cell flattening and a reorganization of the actin cytoskeleton[7–9]. Later findings further supported a function for matrix receptors in coupling the ECM with the actin cytoskeleton. Actin filaments at the cell surface were found to colocalize with extracellular fibronectin and stress fibres were observed to terminate at adhesion plaques[10,11]. Furthermore, disruption of the actin cytoskeleton with cytochalasin B was shown to release fibronectin from the cell surface[12]. It was proposed that fibronectin participates in the formation of attachment plaques linked to the actin cytoskeleton[10,11].

Identification and cloning of adhesion receptors

As protein components of the ECM (for example, fibronectin, vitronectin, collagen) were identified and purified, it was discovered that a short peptide motif, Arg-Gly-Asp, could mediate the cell binding activity of fibronectin and several other ECM proteins[13,14]. This finding, indicating that the binding activity of large insoluble ECM proteins could be reduced to a tripeptide sequence, strongly supported the idea that matrix proteins interact with cells through specific receptors. Efforts were then directed towards the identification and cloning of the membrane receptors for these ECM proteins. Parallel studies in leukocytes focused on the identification of the cell surface proteins recognized by antibodies that block cell–cell adhesion, and studies in platelets sought to identify the fibrinogen receptor. Although it was initially difficult to sort out the molecular nature of the adhesion receptors, it was eventually determined that they consisted of a complex of two membrane proteins, which were termed the α and β subunits.

cDNA cloning of the receptors for fibronectin[2,15], vitronectin[16], platelet fibrinogen[17,18], and lymphocyte adhesion[19,20] demonstrated that these different types of adhesion receptors belonged to a family of homologous membrane proteins, which

were termed 'integrins'[2]. By 1987, it became clear that integrins constitute a widely expressed family of adhesion receptors that represent the major receptors through which cells attach to matrix, and the principle mediators of platelet and leukocyte aggregation and endothelial adhesion[21]. Additional integrins were cloned during the next ten years and alternate splice variants of many integrins were identified, adding further complexity to our understanding the family[1,22].

cDNA clones and antibody probes of integrins provided reagents to probe connections between these receptors and the actin cytoskeleton[1,14]. The β_1 integrin subunit was first shown to colocalize with extracellular fibronectin and several intracellular cytoskeletal components, including actin, α-actinin, vinculin, and talin[23,24]. It was subsequently demonstrated that two actin binding proteins, talin and α-actinin, directly associate with the cytoplasmic tail of the β_1 integrin[25,26]. These findings confirmed the link between integrins and actin filaments and identified some of the proteins involved in this coupling. The observation that talin and α-actinin bind other cytoskeletal proteins, such as zyxin, paxillin and vinculin, which in turn bind tensin, resulted in the proposal that the complex of integrin-linked cytoskeletal proteins in focal adhesions are important for maintaining strong cell–substrate adhesions and promoting cell spreading[27]. The protein components of focal adhesions, and their interconnections that were identified in these early studies, are shown (Fig. 1, top).

Early evidence for integrin transduction of signals

As the connections between integrins and cytoskeletal proteins were being characterized, evidence began to accumulate that integrins could not only regulate actin cytoskeletal rearrangements, but also modulate gene expression and cell differentiation. Attachment to the ECM was found to either induce gene expression in the absence of other factors, or to be a critical requirement for the induction of genes involved in differentiated cell functions[28–31]. In addition, antibodies to β_1 integrin were shown to block the differentiation of myoblasts[32] and fibronectin inhibited the differentiation of keratinocytes[33].

The involvement of integrins in regulating gene expression and cell differentiation motivated investigators to identify the cytoplasmic proteins involved in transducing the signals required for these events. The evidence that v-Src, an oncogenic tyrosine kinase, localizes to focal adhesions, and that focal adhesions could be immunostained with antibodies to phosphotyrosine, provided the first hints of a connection between integrins and tyrosine phosphorylation[34,35].

However, the first direct evidence for integrin-mediated regulation of tyrosine kinases came from studies in platelets, where activation by agonists results in a rapid and strong induction of tyrosine phosphorylation that is predominantly induced by fibrinogen binding to the integrin receptor $\alpha_{IIb}\beta_3$ (refs 36,37). Because tyrosine kinases were first identified in association with oncogene products, such as v-Src and v-Abl, and subsequently with several growth factor receptors[38], there was speculation that this class of protein kinases may specifically regulate cell proliferation pathways. The finding that platelet activation and integrin receptors trigger the activation of tyrosine kinases broadened the function of these kinases to include events triggered by adhesion receptors in differentiated, post-mitotic cell functions. Integrins were also found to regulate sodium–proton antiporters (which mediate changes in intracellular pH)[39,40], and protein kinase C (PKC) was shown to associate with integrin-containing focal adhesions[41].

A major breakthrough in integrin-mediated intracellular signalling came from the identification and cloning of the protein tyrosine kinase, FAK (focal adhesion kinase; a protein with a relative molecular mass (M_r) of 120,000 (120K)). FAK, originally identified as a v-Src substrate[42], was found to localize to focal adhesions and to be inducibly tyrosine phosphorylated after the attachment of cells to ECM proteins, or of platelets to fibrinogen[43–47]. FAK, in common with the sodium–proton antiporter and PKC, was found to be activated by growth factors and other agonists, in addition to integrins. These findings provided significant support for the concept that integrins are indeed signalling receptors, as well as mediators of cell adhesion[1].

During this period, several studies suggested that integrins not only share targets with other receptors, but also cooperate with them in regulating cell behaviour. The engagement of multiple lymphocyte integrins was found to strongly enhance cell proliferation induced by crosslinking of the T cell receptor[48–52], and the adherence of neutrophils to integrin ligands was shown to costimulate the respiratory burst[53]. In addition, simultaneous engagement of both integrins and growth factor receptors was found to enhance activation of the sodium–proton antiporter[39,40].

Inside out signalling

As the signals that are transduced by integrins from the outside of the cell to the inside were being elaborated, parallel investigations on the regulation of integrin affinity demonstrated that information also flowed in the opposite direction through integrins – from integrin cytoplasmic tails to the extracellular ligand-binding domain.
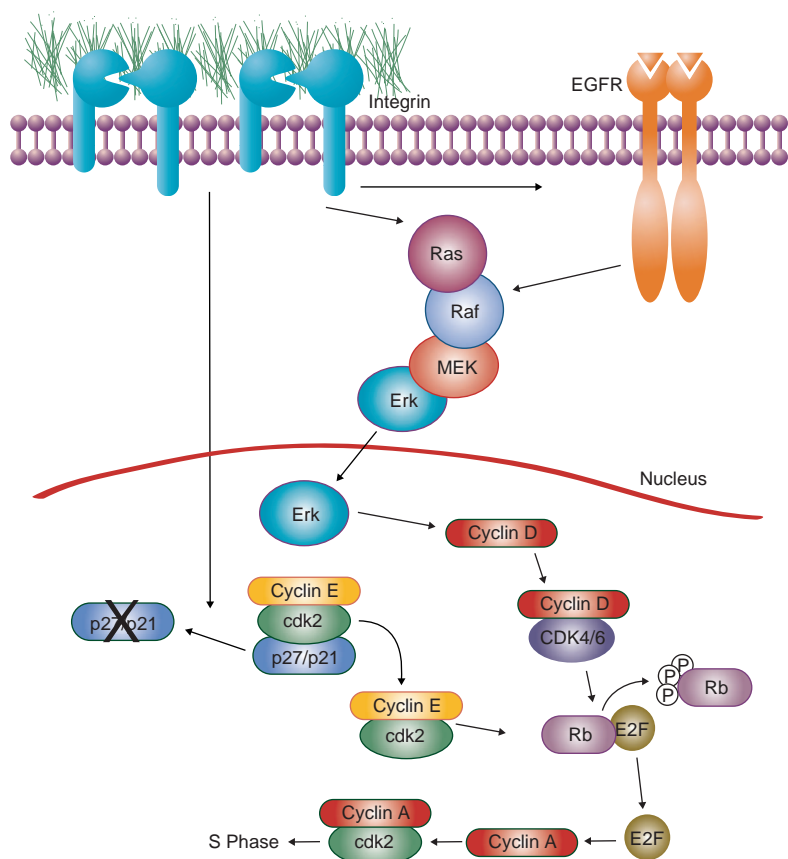
Figure 2 Integrin and growth factor cooperation in cell cycle regulation. Integrins and growth factor receptors cooperate at several levels to ensure the proper control of cell proliferation (for reviews, see refs 137,138,140,141). Both growth factors and cell adhesion are required for transmitting signals to the Ras/Raf/Mek/Erk signalling pathway. Activation of Erk and its nuclear translocation results in an increase in cyclin D1 transcription, and requires growth factor receptor and integrin signals. Cyclin D translation is also controlled through another pathway that results in Sos/PI(3)K-dependent activation of Rac, which is required for the efficient translation of cyclin D1 mRNA[142] (data not shown). *Cyclin D* expression induces the activation of cdk4/6 kinases. Integrins also function to stimulate a degradation pathway that specifically degrades the negative cell cycle regulators p21 and p27, allowing the activation of cyclin E–cdk2 complexes. Activation of both cdk4/6 and cdk2 are required for hyperphosphorylation of retinoblastoma (Rb), causing the release of E2F, which is required for increased transcription of cyclin A. Cyclin A–cdk2 complexes are required for entry into S phase.

This was first suggested by studies before the molecular definition of integrins. Bennett and coworkers found that the binding of fibrinogen to platelets is subject to rapid reversible regulation by agonists[54]. Later studies showed that this regulation was not dependent on the recruitment of receptors to the surface, rather to an increase in the binding activity of the receptor, and that leukocyte adhesion receptors were subject to a similar regulation of ligand binding[55,56]. As integrin family receptors were defined, it became clear that most leukocyte and platelet integrins, including β_1-containing integrins, exist in a resting state until activated by stimuli, and that multiple agonists could regulate individual integrins (for reviews, see refs 57–61). This rapid regulated response prevents the spontaneous adhesion of platelets and leukocytes within the circulation or to the blood vessel wall. The use of biophysical analyses and antibodies that specifically recognize the activated state of β_1, β_2 and β_3 integrins demonstrated that their activation involves alterations in integrin conformation[62–66] and that the cytoplasmic tails of integrins regulate ligand binding activity[67]. Signals from a G-protein, phosphatidylinositol-4,5-bisphosphate (PtdIns(4,5)P$_2$) hydrolysis and PKC were implicated in this regulation[56,68,69]. However, the specific mechanisms involved were unclear.

Thus, by 1992, several paradigms were emerging with regard to the mechanisms involved in integrin-mediated regulation of cell behaviour, namely, that integrins couple with the cytoskeleton, that signals are transduced bidirectionally through outside-in and inside-out mechanisms, and that reciprocal crosstalk between integrins and other receptors regulates responses elicited by any of these receptors. In the decade that followed, much effort was devoted to identifying the cellular components that regulate inside-out and outside-in signalling. These studies not only provided a better mechanistic understanding of how integrins regulate cell behaviour, but also revealed a level of complexity that precluded the generation of simple models to describe pathways leading from integrins to the cytoskeleton or to activation of specific alterations in cell physiology.

Since 1992, the number of signalling proteins linked to integrin activation (by association in focal adhesions or the regulation of activity) expanded to include an overwhelming collection of molecules. These include enzymes such as the Src family kinases, Abl, Syk/ZAP, Csk, Ras, Raf, Mek, Erk, phosphatidylinositol-3-OH kinase (PI(3)K), PKC, Jnk, Cbl, Pyk2 (a homologue of FAK), protein kinase A, Etk, Ack-2, LAR and PEST, as well as adaptor proteins like Crk, Nck, Grb-2 and many others[70–73]. The mechanism by which these proteins are activated, how they couple with each other, and how their activation by integrins affects different cell functions are still under investigation.

Studies of FAK illustrate one example of how networks of proteins are organized downstream of integrins[74]. Integrin clustering and actin polymerization are required for FAK activation, which results in autophosphorylation at a docking site for the recruitment of SH2-containing proteins such as Src or Fyn, the p85 subunit of PI(3)K or PLCγ[75–78]. Src mediates phosphorylation at other sites on FAK, creating additional SH2-domain binding sites[79,80]. Protein binding to these and other sites on FAK results in cascades of protein interactions that transduce signals to many downstream pathways, including Ras/Erk, PI(3)K/Akt, and Crk/Dock180/Rac[81–83]. Thus, integrin engagement, and the subsequent interactions with the actin cytoskeleton, results in the activation of FAK and a host of downstream signals.

Other scaffolding proteins, such as Vav family members, which are activated by integrins in haematopoietic cells, are capable of driving the activation of multiple signalling pathways downstream from integrins[84,85]. In addition, integrins can activate growth factor receptor kinases, and thus trigger the activation of signalling pathways parallel to those involving FAK and Vav[86–89]. Thus, integrins can induce multiple parallel intracellular signalling pathways. However, it is likely that these pathways interact and coordinate with each other, and contribute
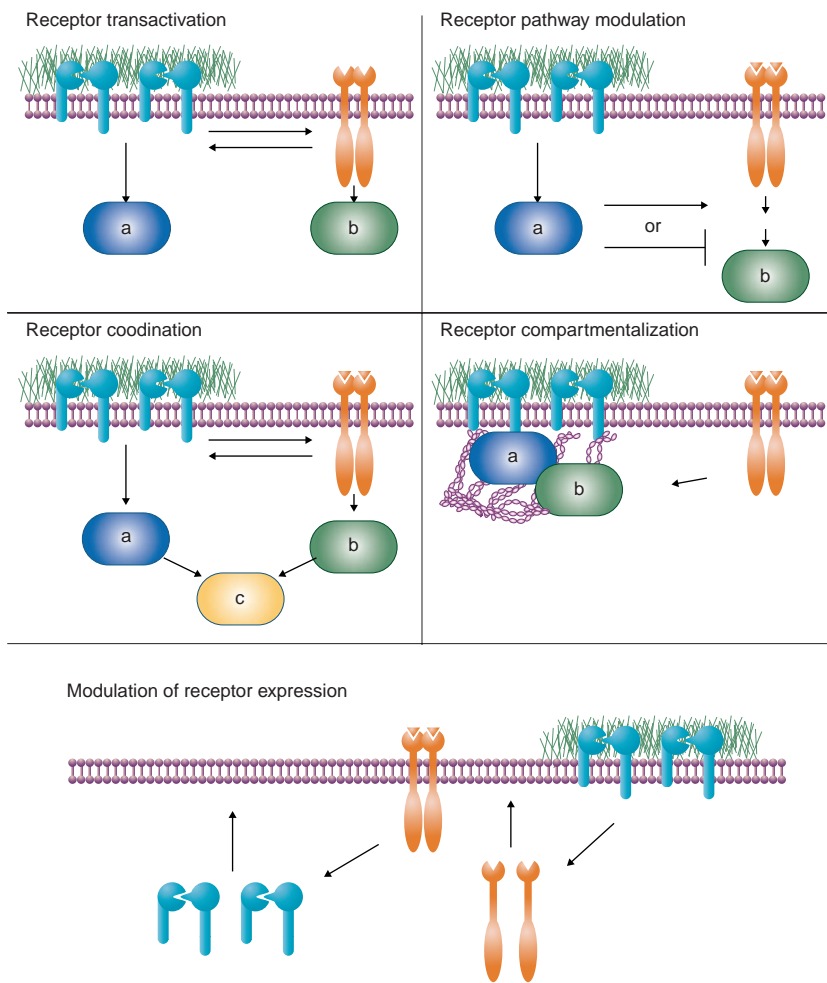
Receptor transactivation

Receptor pathway modulation

Receptor coodination

Receptor compartmentalization

Modulation of receptor expression

Figure 3 **Mechanisms of integrin and receptor cooperation. a, Receptor transactivation. Integrin engagement results in activation of the receptor, or *vice versa*. b, Receptor coordination. The integrin and another receptor each activate a unique signal, both of which are required for the activation of a downstream event. c, Receptor pathway modulation. Activation of a signalling event by integrins either enhances or inhibits signalling mediated by other receptors. d, Receptor compartmentalization. Integrin engagement organizes the signalling components involved in other pathways. This organization is required for the efficient activation of the signalling components stimulated by the other pathway. e, Receptor expression modulation. Integrins, or other receptors, can enhance the expression levels of each other. This can be either a unidirectional response, where activation of one results in upregulation or downregulation of the other, or a reciprocal interaction, where each receptor regulates the level of the other.**

to the complexity of integrin signalling.

Current evidence suggests that multiple pathways are utilized by integrins to activate specific signalling proteins. This is best illustrated for Erk activation[90]. Although FAK is capable of activating Erk mitogen-activated protein kinase (MAPK) through the recruitment of Grb2 (ref. 79), Shc or Src[91], other mechanisms that result in Erk activation have also been described. These include: integrin coupling with caveolin and the recruitment of Fyn, resulting in Shc phosphorylation[92,93]; transactivation of the epidermal growth factor (EGF) receptor by integrins[89]; a Src- and Syk- mediated pathway to

Vav1 (ref. 84), and a Rap1 pathway through B-Raf[94]. These findings indicate that there are multiple, possibly parallel and intersecting, pathways that result in Erk activation through integrins. These findings further highlight the complexity of integrin signalling.

Integrin–cytoskeletal connections

Although many different types of adhesion structures have been characterized, focal adhesions have been most amenable to analysis, as they are relatively stable and are detected in many cultured cells. To date,

more than 50 proteins have been shown to localize transiently or stably in focal adhesions[71,95]. Ligand binding, integrin aggregation and actin polymerization differentially affect the recruitment of distinct focal adhesion proteins[86]. Most focal adhesion proteins contain multiple domains that can individually link with other proteins within focal adhesions, creating webs or networks of proteins in which it is difficult to trace the sequence of events associated with their assembly, or the transduction of signals from the integrins.

A significant breakthrough in this area came with the realization that a family of small GTPases, including Rho, Rac and Cdc42, were involved in inducing actin polymerization and the formation of focal complexes, lamellipodia and filopodia[96,97]. Rho was the first family member implicated in integrin signaling[98]. However, it was later shown that integrin engagement results in the GTP-loading of Rho, Rac and Cdc42 (refs 99–101) and that integrin attachment to the ECM is sufficient for the induction of lamellipodia, filopodia and focal adhesions through Rho GTPases[102,103]. Activation of these GTPases is now regarded as a critical event in integrin-mediated regulation of cell adhesion, cell spreading and cell motility[104]. Several proteins in focal adhesions and lamellipodia can bind actin (for example, α-actinin, talin, tensin, filamin and Vasp/Ena) and are likely to have functions in organizing different types of actin cytoskeletal structures. However, further work will be required to understand which sets of proteins coordinate to generate distinct actin structures[71].

Studies of FAK also illustrate the intimate relationship between integrin-regulated signalling proteins and the cytoskeleton (Fig. 1, bottom). Although FAK is not required for focal adhesion formation[105,106], FAK activation by integrins and growth factors is dependent on actin polymerization and actomyosin contractility[47,107]. These cytoskeletal processes may be critical to induce the clustering of FAK required for its activation. FAK is also able to recruit and bind cytoskeletal proteins such as paxillin and talin, and to activate Rho GTPases through FAK binding proteins like Cas, which link to Rac through the interaction of Crk with Dock180 (ref. 81). These events may be important for focal adhesion turnover and cell migration, as *FAK*-null fibroblasts are defective in these processes[105]. Thus there is a two-way communication between the cytoskeleton and intracellular signalling pathways.

The cytoskeleton also has a function in regulating inside-out signalling events. The activation of β_2 integrins involves changes in avidity through clustering and the increased diffusion of β_2 in the membrane[108,109], in addition to conformational changes[110–112]. The clustering of integrins is

regulated by signalling enzymes like PI(3)K, PKCs, and the Ras and Rap GTPases (for reviews, see refs 113,114), as well as adaptor proteins like SLAP 130/Fyb[115,116], and seems to involve changes in actin cytoskeletal structures that allow the lateral movement of integrins[114,117,118]. Thus, as with outside-in signalling, intimate interactions between signalling proteins and the cytoskeleton are important for regulating integrin avidity by inside-out signalling.

Whereas most integrins couple with actin-containing cytoskeletal structures, $\alpha_6\beta_4$ integrin was found to be unique in its structure and localization. $\alpha_6\beta_4$ integrin is expressed exclusively in epithelial cells, where it specifically localizes to hemidesmosomes[119–121]. It couples extracellular laminin to intermediate filaments through the protein plectin (for reviews, see refs 122,123). Hints that signals may be transduced through these adhesive structures are just emerging.

Integrin interacting membrane proteins

Several membrane proteins, referred to as integrin-associated proteins, stably couple with integrins and modulate integrin responses. These include CD47 (a pentaspanin protein), tetraspanin proteins (TM4SFs), growth factor receptors (for example, platelet-derived growth factor (PDGF), insulin, and EGF receptors), syndecan heparin sulfate proteoglycan receptors, glycosyl phosphatidylinositol (GPI)-linked receptors (for example, urokinase plasminogen activator receptor, CD98), CD36 and CD46 (for reviews, see refs 124–128). These proteins have been shown to regulate intracellular signalling and control integrin adhesion, migration, invasiveness and matrix assembly. A description of the specific signals regulated by these receptors is beyond the scope of this review. However, several specific examples of crosstalk are cited throughout.

ECM presentation

It is now clear that responses to integrin engagement are dependent not only on which ligand is bound to a specific integrin, but also the form in which the ligand is presented to the integrin. For example, prolactin-induced transcription of β-casein by mammary epithelial cells is highly dependent on which matrix protein they are attached to, as well as whether the matrix is immobilized on plastic or in a gelatinous state[28,29,129]. Prolactin-induced phosphorylation of its receptor was found to be blocked (possibly because of phosphatase activity) when mammary epithelial cells were cultured on immobilized collagen, but not on laminin. This indicates that integrins control an early step in signal transduction mediated by prolactin, possibly by providing 'permissive' signals to allow receptor signal transduction[130]. Perhaps one of the most dramatic demonstrations of the importance of basement membrane–integrin interactions in regulating cell behaviour was the observation that manipulations of β_1 integrin complexes can cause tumorigenic mammary cell lines cultured in basement membrane gels to undergo a phenotypic reversion, such that they form structures resembling normal mammary acini[131,132].

Recently, it was shown that plating fibroblasts on three-dimensional matrices results in the formation of novel focal adhesion structures that had not been detected when cells are grown on immobilized matrix proteins[133]. The differences between the two- and three-dimensional structures were attributed to differences in the pliability and rigidity of the matrices under the two different conditions. These results, together with other studies using flexible matrices, indicate that differences in mechanical tension can regulate cell adhesion complexes, cell shape, polarity and the expression of differentiated cell functions[71,134–136].

In the sections below, we use several examples to illustrate our current understanding of the molecules that transduce signals from integrins, the intimate coordination of integrin signals with other cellular receptors, and how signalling pathways are organized into networks, rather than linear pathways.

Integrin control of cell proliferation

In studies over the past 10 years, investigators have attempted to define which steps in cell cycle progression are dependent on attachment to the ECM. These studies demonstrated that there is not a single 'checkpoint' that monitors cell adhesion status; rather, there are multiple steps in cell cycle progression that require matrix attachment[137,138]. It was first shown that the induction of cyclin A production is blocked in suspended cells treated with growth factor[139]. Later, the activation of several cyclin-dependent kinases (Cdks) involved in G1 phase progression and S phase initiation were found to be controlled through multiple integrin-dependent events (Fig. 2). These include the induction and translation of cyclin D and cyclin A, as well as p27[cip1] and p21[kip1] degradation[137,138,140–142]. Integrins control these events through several mechanisms, including the enhancement of growth factor signals, the recruitment of proteins to membrane/cytoskeletal complexes, or the enhancement of nuclear translocation. Many of these regulatory events involve both transcriptional and post-transcriptional controls.

It is clear that signals from integrins intimately coordinate with pathways activated by growth factors at multiple steps during cell proliferation. A prediction from these findings is that the acquisition of anchorage independent proliferation would require an oncogene capable of activating all the integrin-dependent events required for cell cycle progression[143]. Indeed, oncogenes like Ras and v-Src function at early steps in growth factor- and integrin-stimulated pathways, thus affecting the critical downstream signals triggered by integrin adhesion. For example, oncogenic Ras variants can rescue the defects in Cdk activation, p21 and p27 degradation, and Erk, Akt, Rac and sodium–proton antiporter activation, induced by placing cells in suspension.

Although the studies described above infer that signals from integrins regulate proliferation in a dose-dependent fashion, experiments addressing the relationship between the number of adhesive contacts and the regulation of signalling and cell behaviour suggest a non-linear relationship and additional levels of control. Varying the extent of spreading (that is, cell shape), without changing the adhesive contact area of cells, was found to govern the proliferative capacity of cells and cell survival[144]. Erk activation by growth factors does not vary with spreading. However, cyclin D expression and downregulation of p27[kip] is defective in poorly spread cells. These studies suggest that certain signalling events are regulated by cell shape and provide a molecular explanation for previous reports indicating that cell shape and surface area are critical determinants of cell proliferation[5,145]. In addition, they suggest that shape-sensitive sensors (possibly involving tension and cytoskeletal constraints on intracellular processes) have an important function in regulating signal transduction through integrins.

Integrin control of cell survival

The importance of integrin adhesion in cell survival was first demonstrated in endothelial and epithelial cells, where detachment from the ECM results in apoptotic cell death, even when cells were incubated in normal levels of serum[146,147]. Apoptosis induced by cell detachment has been referred to as anoikis (the Greek word for homelessness)[146]. Although a function for integrins in survival was identified through cell detachment studies, it is now clear that integrins can protect cells from death induced by other death stimuli (for example, chemotherapeutic agents and serum withdrawal[148,149]). Many proteins implicated in the regulation of cell survival by other receptors are activated by integrin adhesion, and detachment from the ECM results in their inactivation. These include the PtdIns(3,4,5)P$_3$-activated kinase Akt/PKB[150,151], FAK[152] and the Ras/Erk pathway[153] (for a review, see ref. 151). Integrins

regulate cell survival through the inhibition of pro-apoptotic proteins and the expression of anti-apoptotic proteins, such as Bcl-2 (ref. 149), Flip[154], and IAPs[155]. Conversely, detachment from the ECM results in the activation of pro-apoptotic proteins, such as Bax[156], caspases[157,158] or the death ligands Fas or Trail[154], and inhibition of anti-apoptotic proteins.

Recent studies of Bim and Bmf, two pro-apoptotic Bcl-2 family proteins that contain only BH3 domains, indicate that they may function as intracellular sensors of the cytoskeleton and the state of ECM attachment. These proteins are bound to isoforms of the dynein light chain, which associates with either microtubules or microfilaments[159,160]. Detachment from the ECM or inhibition of actin polymerization results in the dissociation of Bmf from dynein light chain and its relocation to the mitochondria, where it functions as a pro-apoptotic protein. Bim expression is induced by downregulation of Akt, so induction of *Bim* expression may also be involved in anoikis[161].

The ability of integrins to protect cells from apoptosis is both integrin- and cell-specific. For example, primary mammary epithelial cells treated with insulin are protected from cell death when plated on laminin, tenascinC or collagen IV, but not on collagen I (refs 162,163). The protective effects of insulin are dependent on the ability of the integrin to promote activation of Akt/PKB through the insulin receptors. In CHO cells, $\alpha_5\beta_1$, $\alpha_v\beta_3$ and $\alpha_1\beta_1$ integrins protect cells from anoikis, whereas $\alpha_v\beta_1$ does not provide this protection[149]. Protection from death correlates with the ability of the integrin to induce *Bcl-2* expression (ref. 164).

Lastly, recent studies indicate that unliganded integrins can also induce cell death under certain conditions[165]. This type of cell death is distinct from anoikis, as it can take place under conditions where other integrins within the same cell are ligated.

Integrin crosstalk with other receptors

In the sections above, multiple examples were cited where integrins can regulate the responses to other receptors, and *vice versa*, where integrins are regulated by other receptors. Thus, there is extensive crosstalk between pathways activated by integrins and other receptors. In the section below, we describe several different types of crosstalk that have been demonstrated. The examples below represent an oversimplification of complex interactions. However they are useful in considering the functional importance of pathway crosstalk (Fig. 3). In most of the examples provided, the isolated interactions represent only one aspect of multilevel coordinated control:

First, receptor transactivation. The most striking example of this type of crosstalk is integrin activation of growth factor receptors. EGFR, PDGFR, vascular endothelial growth factor receptor (VEGFR), hepatocyte growth factor receptor (HGFR; Met), and Ron are all activated after the engagement of integrins[86–89,166,167]. Integrin-activated growth factor receptors are capable of amplifying integrin signals. Shc/Erk activation in several cell types is dependent on integrin-induced EGFR activation, and adhesion-induced cell survival mediated through PI(3)K also requires ECM activation of EGFR[89]. The ability of cell adhesion to activate the HGF receptor (Met) is crucial for tumour metastasis in a hepatocyte tumour model[168]. As discussed above, growth factors and other agonists can activate integrins through changes in integrin affinity and avidity.

Second, receptor coordination. In this type of crosstalk, each of two or more receptors contributes components that are necessary for the activation of an intracellular event. One of the earliest examples of this involves a situation where integrins provide a substrate for an enzyme activated by a growth factor. Reduced levels of inositol-1,4,5-trisphosphate $(Ins(1,4,5)P_3)$ induction after stimulation of suspended cells with PDGF results from a lack of the substrate $PtdIns(4,5)P_2$, caused by a loss of PtdInsP5 kinase activity in detached cells[169]. Coordination is also observed between integrins and syndecan proteoglycan receptors, which both interact with ligands on fibronectin[170]. Signals from syndecans influence integrin-mediated focal adhesion assembly through PKC, Rho, and syndesmos (a paxillin binding protein)[171–173].

Third, receptor pathway modulation. In this type of crosstalk, a signal from one receptor provides a costimulatory or inhibitory signal to another receptor pathway. There are numerous examples of such regulation relating to the regulation of Erk activation and cell cycle progression[70,72,137,138]. For example, it has been shown that integrin signals are required for growth factor activation of Erk. Although Ras is activated by growth factors independently of integrin engagement, Raf or MEK activation is dependent on integrins[174,175].

Fourth, modulation of receptor expression. This mechanism involves the induction or repression of receptor expression by another receptor. Growth factor receptor enhancement of motility in several cell types results from the upregulation of integrin receptor expression[176]. An interesting reciprocal regulation of integrins and growth factor occurs in mammary epithelial cells cultured in three-dimensional basement membrane gels, where they organize into polarized structures that resemble glandular acini *in vivo*[134].

Manipulations that lower either *EGFR* or β_1 *integrin* expression in these three-dimensional gels, but not in two-dimensional culture, were found to cause downregulation of the other receptor[132]. These results suggest that integrins and growth factors couple in distinct ways, depending on the context in which the cells are cultured

Fifth, receptor compartmentalization. In this type of crosstalk, the activation of one receptor induces the formation of scaffolds, or structures that organize signalling components of other receptors. It is likely that the cytoskeletal assemblies induced by the attachment of integrins to ECM have an important function in organizing the signalling pathways of other receptors. However, the precise nature of the interactions has not, as yet, been elucidated. For example, growth activation of FAK, paxillin and PAK, as well as P2Y nucleotide G protein-coupled receptor (GPCR) activation of Erk, are all inhibited by detachment from the ECM or treatment with inhibitors of actin polymerization[99,177,178]. It is likely that integrin–cytoskeletal complexes are critical to link growth factor receptors and GPCRs to these signalling proteins, but the nature of these links are not currently understood.

In vivo functions of integrins

Integrins have been implicated in many cellular functions through the *in vitro* studies discussed above. However, recent studies analysing integrin mutants in worms, flies and mice have provided important information on integrin function *in vivo*. In *Drosophila melanogaster* and *Caenorhabditis elegans*, integrin mutations cause defects in multiple developmental events, including the expected alterations in the attachment of cells within and between tissues, but they also cause defects in dorsal closure, endoderm migration and tubulogenesis[179,180]. In mice, disruption of β_1 integrin, which is a subunit of at least 12 integrins, causes peri-implantation lethality[181]. Analysis of chimaeric mice that lack β_1 integrin in a subset of cells or tissues, and mice lacking other β or α subunits, have identified more specific defects in many processes, including haematopoiesis, haemostasis, immune defenses and the migration of several cell types, neural organization, organ development, the formation and maintenance of vasculature, and the integrity of skeletal and cardiac muscle, skin, bone and cartilage[180,182]. It is difficult to establish whether processes regulated by integrins *in vivo* require intracellular signal transduction, or merely extracellular adhesive functions; however, genetic ablation of integrin genes in mice (but not in flies) suggest a requirement for integrins in regulating proliferation of certain cell populations, including keratinocytes, dermal fibroblasts, mammary and intestinal epithelial cells[182]. Cell survival

defects have also been observed in some integrin-null mice[182].

In humans, a lack of the platelet integrin $\alpha_{IIb}\beta_3$ or the β_2 leukocyte integrin subunit result in diseases associated with bleeding and recurrent infections, respectively[183–185]. Mutations in the β_4 subunit cause a severe skin blistering disease, epidermolysis bullosa, caused by defects in attachment to laminin. Mutations in several ECM proteins are also associated with muscular dystrophy and skeletal malformations[186,187]. Escape from cell death and proliferative suppression resulting from loss of normal adhesive interactions is likely to be involved in cancer progression (for a review, see ref. 188).

Conclusions

As discussed above, studies using a wide array of different approaches have revealed the importance of integrins in regulating cell behaviour, and have provided insights into some of the mechanisms responsible for integrin functions. One conclusion from this body of work is that integrins seem to function as 'sensors' that survey the extracellular space and respond by transducing signals that directly regulate cell behaviour by modulating responses to other extracellular stimuli. This sensing function of integrins may ensure that cellular activities take place only when a cell is appropriately situated in its physical environment. The ability of integrins to sense and modulate the cell environment involves many complex interactions and levels of regulation, only some of which we have begun to grasp.

Signal transduction pathways, once visualized as linear biochemical interactions, neatly progressing from one step to the next, are now understood to comprise complex webs of interconnected pathways. Studies of integrins have greatly expanded our understanding of the nature of these networks, especially the importance of cytoskeletal interactions and crosstalk between receptor pathways. These studies have also highlighted the variation in signal coupling in different cell types and within different ECM microenvironments. We are now faced with defining signalling events in terms of specific cell contexts, further increasing the complexity of our understanding of how receptors regulate cell behaviour. Although this perspective focused on ECM interactions mediated by integrins, cell–cell interactions and other ECM receptors also have important functions in modulating cellular responses. In the future, more effort should be invested in examining cultured cells under conditions where cell–cell and cell–matrix interactions more resemble their 'native' environment. □

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