CMLS, Cell. Mol. Life Sci. 62 (2005) 1081–1099
1420-682X/05/101081-19
DOI 10.1007/s00018-005-4522-8

CMLS Cellular and Molecular Life Sciences

Review

Integrin-actin interactions

S. Wiesner *, K. R. Legate and R. Fässler

Max-Planck-Institut of Biochemistry, Department of Molecular Medicine, Am Klopferspitz 18, 82152 Martinsried, (Germany), Fax: +49 89 8578, e-mail: wiesner@biochem.mpg.de

Received 24 November 2004; received after revision 17 January 2005; accepted 19 January 2005
Available online 09 March 2005

Abstract. The integrin family of extracellular matrix receptors regulates many aspects of cell life, in particular cell adhesion and migration. These two processes depend on organization of the actin cytoskeleton into adhesive and protrusive organelles in response to extracellular signals. Integrins are important switch points for the spatiotemporal control of actin-based motility in higher eukaryotes. Ligands of integrin cytoplasmic tails are central elements of signalling pathways involving small GTPases as well as protein and lipid kinases in the regulation of F-actin crosslinking, actin treadmilling and de novo nucleation of actin filaments. We present an overview of common pathways and discuss recent evidence for their differential use by individual integrin receptors.

Key words. Integrin; actin; motility; adhesion.

Introduction

To maintain the integrity of multicellular organisms, cell shape and motile behaviour must be tightly controlled. To this end, higher eukaryotes have developed mechanisms that regulate cell adhesion and motility in response to extracellular signals. The analysis of these mechanisms is central to the understanding of many biologically and clinically important processes, such as the creation of new tissues and organs during embryogenesis, the coordinated movement of keratinocytes and fibroblasts during the closure of skin wounds and the extravasation of immune cells during inflammation. Over the last 10 years, enormous progress has been made towards understanding the molecular machinery that cells use to change their shape and move [1]. The combined use of genetic, biochemical and biophysical approaches has identified molecules and mechanisms which appear to be common to almost all animal cells. Basic building blocks of cell protrusions show remarkable evolutionary conservation, in particular the actin cytoskeleton and key actin binding proteins such as the actin-nucleating Arp2/3 complex, the formins and their respective activators. Biochemical and biophysical models have been developed which can account quantitatively for many aspects of cell migration. Simultaneously, the Rho family of small molecular weight GTPases have emerged as pivotal control elements of protrusive and retractive cell movements. Motile cell behaviour is influenced by a complex interplay of signals induced by chemical messengers such as growth factors, by cell-matrix and cell-cell interactions, and by cell-autonomous regulation.

The integrin family of cell surface receptors plays a central role in the transduction of cell-matrix adhesion signals. Integrin signalling contributes not only to cell adhesion and migration, but also regulates cell proliferation, survival and differentiation. The actin cytoskeleton is one of the most important targets of integrin-mediated signalling. Many of the molecules that transmit signals from integrins to the actin cytoskeleton have been identified; we are now faced with the challenge of explaining how their manifold interactions with each other, with integrins, with effectors of the actin cytoskeleton and with

* Corresponding author.

other signalling pathways work together to achieve the astounding specificity of integrin-mediated signalling. This review focuses on common mechanisms of signal transduction from integrin cytoplasmic tails to the actin cytoskeleton and discusses recent evidence for differential use of these signalling pathways by different classes of integrin receptors.

Given the high tissue and cell type specificity of integrin signalling and its far-reaching consequences for cell fate, it is important to emphasize that the following description of integrin-actin interactions and regulatory mechanisms cannot claim to be universally valid. Much of the available data was obtained from experiments using fibroblastoid cells cultured on rigid two-dimensional (2D) matrices. Few of the derived model pathways have been thoroughly validated in vitro using other cell types and experimental approaches, or in vivo through gene deletions and modifications. The role of integrins in actin-based motility may therefore be somewhat misrepresented. Anchorage-independent growth and motility are increasingly perceived as phenomena that occur not only in transformed cells but also in various normal cell types. For example, T-lymphocytes do not require integrins for migration through three-dimensional (3D) collagen lattices [2]. To avoid confusion, in this review we will mainly focus on data obtained from cell types that display well-characterized anchorage-dependent growth.

Integrins

Integrins are a family of heterodimeric receptors composed of non-covalently linked class I transmembrane α and β subunits. Members of the integrin family have been identified in many diverse organisms from *Drosophila melanogaster* and *Caenorhabditis elegans* to sponges and corals [3–5]. Both vertebrate and invertebrate integrins show conserved structural and functional features. Through the globular head of their extracellular domains, integrins bind components of the extracellular matrix (ECM). The short cytoplasmic tail domains of α and β subunits have no catalytic function. Rather, they transduce signals by binding to a variety of effector proteins that may be either directly or indirectly associated with the actin cytoskeleton. Integrins and their cytoplasmic binding partners are organized into large, highly dynamic multiprotein complexes termed matrix adhesions.

In man, 18 α-integrin and 8 β-integrin subunits have been described which can associate in a heterodimeric fashion to form at least 24 different receptors (table 1; [6–8]). A simplified classification system based on specificity for ECM ligands yields three classes of receptor: RGD receptors that bind to ECM components containing the Arg-Gly-Asp motif (fibronectin, vitronectin), laminin receptors and collagen receptors. Apart from ECM components, some integrins such as the β2 subgroup mediate cell-cell adhesion by binding to counter receptors on other cells. Many integrins also bind to soluble proteolytic fragments of vascular basement membranes such as endostatin [9], and a number of pathogens make use of integrins as receptors for cell entry [10].

Integrins display allosteric regulation in that binding of both extracellular, and intracellular ligands can trigger a transition between a low-affinity conformation (characterized by close association of the cytoplasmic tails and a bent extracellular domain) and a high-affinity conformation (dissociated tails, erect extracellular domain) [7, 11, 12]. The mechanisms underlying these conformational changes have become clearer in the past few years through high-resolution structural analysis of integrin heterodimers in complex with ligand fragments [13–15].

Integrin subunits undergo extensive posttranslational modification that affects their function. The regulation of integrin-ligand binding by phosphorylation, alternative splicing and proteolytic cleavage of integrin cytoplasmic tails is discussed below. Glycosylation of the extracellular domains of α and β subunits is also likely to regulate function [16].

Table 1. The integrin receptor family in man.

β1						β3		β4
β1			β7		β2	β3	β5,6,8	β4
RGD	Laminin	Collagen	RGD	cell-cell	cell-cell	RGD	RGD	Laminin
α5β1								
α8β1		α1β1			αDβ2			
	α3β1						αVβ5	
αVβ1		α2β1			αLβ2	αVβ3		
	α6β1		α4β7	αEβ7			αVβ6	α6β4
α4β1		α10β1			αMβ2	αIIbβ3		
	α7β1						αVβ8	
α9β1		α11β1			αXβ2			

The grouping is based on the probable evolutionary relationship of the β subunits and the main ligands of individual receptors.

Biological functions of integrins

The most basic function of integrins, as that of other adhesion receptors, is to provide a mechanically strong connection between cells and the ECM. This function is particularly evident where integrins mediate attachment to basement membranes. For example, loss-of-function alleles of the *C. elegans* β-integrin subunit βpat-3, which localizes to the basement membrane connecting the body wall muscles to the cuticle, leads to a detachment of muscles from the cuticle following the first contraction [17]. Beyond cell-ECM attachment, matrix adhesions are also involved in the regulation of matrix deposition and degradation, and they are important signalling platforms that recruit proteins regulating small GTPase activity, protein and lipid kinases and adaptor proteins.

The transduction of mitogenic signals through integrins is closely coordinated with growth factor receptor activity (reviewed in [18, 19]). Activated integrins can cause transactivation of a number of receptor tyrosine kinases such as Met [20], platelet-derived growth factor receptor [21], vascular endothelial growth factor receptor [22] and epidermal growth factor receptor [23, 24]. In some cases this involves close physical proximity of growth factor receptors and integrins [25].

Additionally, activated integrins, through their cytoplasmic ligands, can activate a number of extracellular signal-related kinases (ERKs) and mitogen-activated protein kinases (MAPKs) [26–28]. Conversely, binding of integrins to soluble ligands [29] or integrin overexpression [30] can inhibit proliferation and lead to programmed cell death through both caspase-dependent [31] and caspase-independent mechanisms [32]. Dysfunctional integrin-mediated signalling can contribute to cancer progression by promoting invasion and metastasis of neoplastic cells, and tumor angiogenesis [33–35]. Thus, integrin-mediated signalling in response to ECM adhesion acts in concert with growth factor signalling to create a specific microenvironment for cells in a tissue context, and exerts a precise control over cell fate.

The anatomy of matrix adhesions

Matrix adhesions are highly dynamic structures that organize around activated integrin clusters [36]. Many of their protein components (table 2) are essential in higher eukaryotes or lead to severe defects in cell motility when deleted. All subtypes of matrix adhesions are areas of very close contact between the plasma membrane and the

Table 2. Ligands of β integrin cytoplasmic tails.

Protein	Integrin	Function/phenotype	Binding partners	Isoforms
Talin	β1,β2,β3 αIIb	integrin activation/F-actin binding KO: no MA formation	FAK, vinculin F-actin PIP(4,5)2, PIPKIγ	talin-1, talin-2 (H)
Paxillin	β1,β2,β3 α4,α9	adaptor KO: reduced migration rates and MA turnover	FAK, ILK, vinculin, α-parvin PKL, PAK Git, PAG LIM kinase	paxillin α,β,γ (AS) Leupaxin (H) Hic-5 (H)
FAK	β1,β2,β3	kinase, adaptor KO: reduced migration rates and MA turnover	paxillin, talin p130Cas, Src PI3K, PLCγ GRAF, ASAP, p190RhoGEF N-WASP	FRNK (AS) Pyk2 (RAFTK/CAKβ) (H)
ILK	β1,β2,β3	kinase, adaptor KO: delayed MA formation	paxillin PINCH, parvin ILKAP PIP3	Ilk-1, Ilk2 (AS)
α-actinin	β1,β2,β3	F-actin binding	vinculin, zyxin, β-parvin F-actin Erk1/2	α-actinin 1-4 (H)
Filamin	β1,β2,β7	F-actin binding	F-actin Trio migfilin	filamin A,B,C (H)
Tensin		F-actin binding KO: reduced migration		tensin 1,2,3; c-ten (H)
Myosin-X	β1,β3,β5	motor		

MA, matrix adhesions; AS, alternative splicing; H, homologue(s); KO, targeted deletion in mouse.

substrate. The number of adhesions, their morphology and molecular composition vary widely between cell types and also between subcompartments within the same cell. Tremendous progress has been made in analyzing the molecular composition, dynamics and cell type specificity of matrix adhesions.

The current nomenclature differentiates between focal complexes, focal adhesions, fibrillar adhesions and podosomes. Focal complexes are small, dot-like matrix adhesions found within spreading or migrating cells and are usually concentrated in membrane protrusions (size around 100 nm). They are dynamic structures that either turn over or mature into larger, more elongated focal adhesions (alternatively named focal contacts; size around 1 μm) within a few minutes. Focal adhesions are structures predominantly found in resting cells or areas of cells with low motility and display much slower turnover than focal complexes. They are usually the attachment points of actin stress fibres to the cell membrane. A variant of focal adhesions is the fibrillar adhesions formed by many cell types adhering to fibronectin via integrin $\alpha5\beta1$. They differ from focal adhesions in that they arrange extracellular fibronectin fibrils in a parallel orientation with actin stress fibres, and they do not contain some common focal complex/adhesion components such as vinculin and paxillin [37]. Podosomes are found naturally in osteoclasts or cells of hematopoietic origin such as neutrophils, and represent the only matrix adhesion type found in these cells (recently reviewed in [38]). They compare with focal complexes in both size and half-life, but are composed of a ring-like assembly of matrix adhesion components surrounding an F-actin core. Podosomes are also prominent in v-Src transformed cells [39]. Recently, a related structure has been characterized in various epithelial cell types [40]. Epithelial podosomes are described as structures that are surrounded by a rosette of hemidesomosomes, and share many features with mesenchymal podosomes.

Regulation of the actin cytoskeleton by integrin cytoplasmic tail binding proteins

Matrix adhesions are short-lived and difficult to study as isolated organelles. Therefore, immunofluorescence and video microscopy studies have been most beneficial to our understanding of the molecular mechanisms at work in these structures. We dispose today of several hypotheses to explain how focal complexes form in response to integrin activation, and how their fate bifurcates to signal either activation of actin-based protrusion mechanisms associated with focal complex turnover, or maturation of focal complexes into focal adhesions connected to the cytoskeleton by actomyosin stress fibres. Several proteins in the focal adhesion cytoplasmic plaque are implicated in regulation of the actin cytoskeleton.

Talin and vinculin

Talin is a 270-kDa F-actin binding protein which plays a key role in integrin activation, the initiation of matrix adhesion formation and the linkage of integrin receptors to the actin cytoskeleton (reviewed by [41, 42]). Talin, which self-associates via the rod-shaped C-terminus to form a dimer, is one of the first proteins identified as a cytoplasmic integrin ligand [43]. The globular head of talin contains a four-point-one, ezrin, radixin, moiesin (FERM) domain which binds with high affinity to the cytoplasmic tails of integrin $\beta1$, $\beta2$, $\beta3$ and $\beta5$, but only weakly to $\beta7$ [44]. This interaction involves a conserved NPxY motif in β-integrin tails [45]. The head region also contains binding sites for focal adhesion kinase (FAK) [46], phosphatidylinositol-4,5-biphosphate (PIP_2) [47], phosphatidylinositol-4-phosphate 5-kinase type I γ (PIPKIγ) [48, 49] and for the hyaluronan receptor layilin [50]. The tail region contains an additional low-affinity binding site for β-integrin tails [51] as well as two actin and vinculin binding sites [52]. Binding activity of this region for F-actin as well as G-actin has been demonstrated [53].

Talin plays a central role in the first steps of focal complex formation following initial integrin engagement, and talin degradation plays a critical role in focal adhesion turnover [54]. A first demonstration of this was that microinjection of anti-talin antibodies into chicken dermal fibroblasts led to matrix adhesion disassembly [55]. Since talin-deficient mice die at E8.5–9.5 [56], much of the biological function of talin has been elucidated from cell culture experiments. Talin-1-deficient undifferentiated embryonic stem (ES) cells fail to spread on collagen or laminin; however, these cells can spread on fibronectin but are unable to assemble vinculin- or paxillin-containing focal adhesions, or stress fibres [57]. Talin knockdown in CHO cells was shown to inhibit activation of integrin αIIb$\beta3$, αV$\beta3$ and $\alpha5\beta1$ [58]. Conversely, overexpression of the talin N-terminal head leads to a threefold increase in integrin αIIb$\beta3$ activation in CHO cells [59]. By using optical tweezers to obtain force measurements, talin was identified as the important component for maintaining a 2 pN slip bond between fibronectin and the cytoskeleton [60, 61]. It has been suggested that the mechanistic basis for talin-mediated integrin activation is through disrupting a salt bridge between the α and β cytoplasmic tails [62, 63].

The current model of integrin activation by talin includes a positive feedback loop through increased local PIP_2 production, possibly mediated by the PIP kinase isoform PIPKIγ. PIPKIγ is recruited to matrix adhesions in a complex with talin [49]. PIP_2 binding by talin increases the affinity of the FERM domain for integrin cytoplasmic tails [47]. Both PIP_2 and talin bind to and activate vinculin, an actin binding protein, by relieving autoinhibitory binding of the vinculin head domain (V_H) with

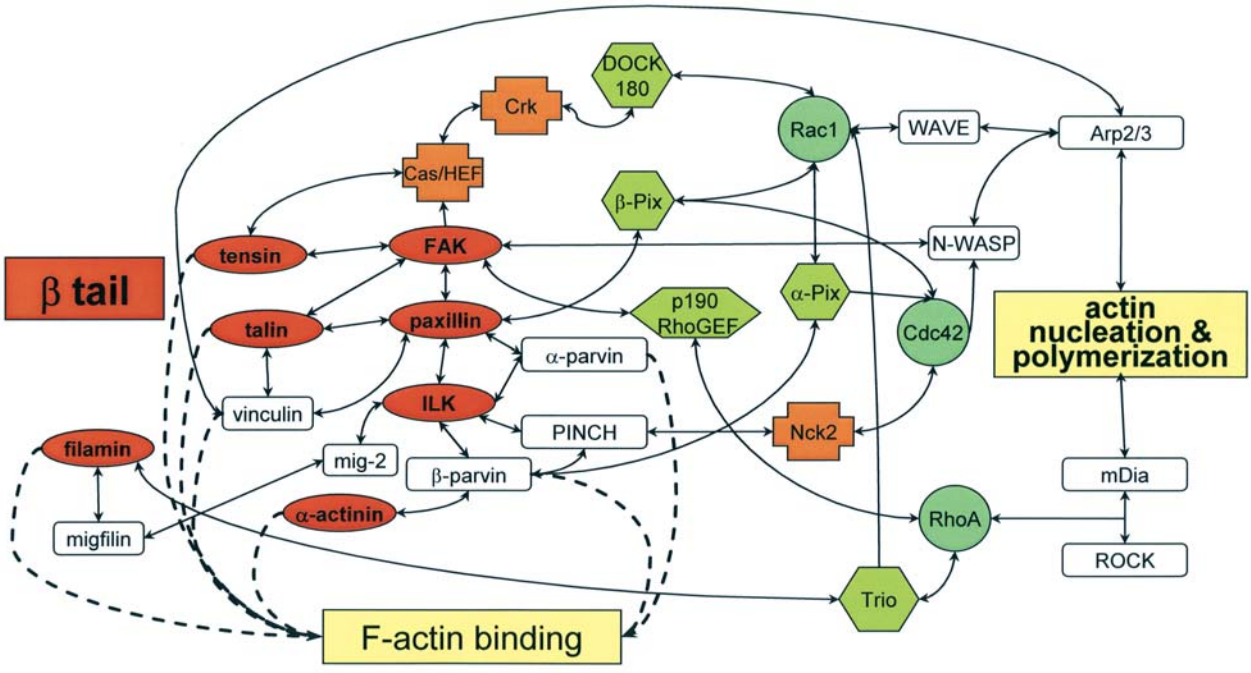

Figure 1. Schematic overview of the interaction between integrin-proximal cytoplasmic proteins. Integrin β cytoplasmic tail and direct ligands are depicted in red; GEFs, yellow-green; small GTPases, bright green; SH3/SH2 adaptors, orange.

the tail domain (V_T) [64]. Vinculin may promote and stabilize initial integrin clustering mediated by talin dimer formation by providing additional F actin crosslinks to the nascent focal complex. A role for vinculin as a negative regulator of matrix adhesion turnover is suggested by reports showing that the migration velocity of vinculin –/– murine embryonic fibroblasts is two times faster than that of wild-type cells [65], and that vinculin localizes preferentially to mature focal adhesions [66]. Thus, the coordinated recruitment of talin, PIPKIγ and vinculin to activated integrins may be considered as a minimal paradigm for how different protein classes work together to form focal complexes, link them to the cytoskeleton and promote their maturation into focal adhesions.

Talin-induced conformational changes of the $\alpha\beta$-tails as well as the presence of talin itself could provide an initial scaffold to assemble a more complex matrix adhesion. Interestingly, a talin homologue is also present in *Dictyostelium discoideum*, an organism which lacks integrins. In this organism talin functions in actin-based motility and adhesion-dependent cytokinesis [67, 68].

FAK

FAK is a non-receptor protein tyrosine kinase that plays a central role in signalling through integrins and a variety of other receptors (reviewed in [69]). The kinase domain is situated in the central part of the molecule and is flanked by large non-catalytic domains. The N-terminus contains a FERM homology domain which binds to peptides of the β1-integrin cytoplasmic tail [70]. The functional relevance of direct FAK binding to integrin is unclear since the integrin-binding region of FAK is not required to target the molecule to focal adhesions [71]. Based on the observation that N-terminal deletion mutants of FAK display increased kinase activity under certain conditions [72], it has been proposed that, in analogy to other FERM domain-containing proteins, FAK is subject to autoinhibition by binding of its FERM domain to its carboxyl terminus, a hypothesis that has recently been strengthened by binding studies using recombinant fragments of FAK [73].

The carboxyl terminus of FAK contains the focal adhesion targeting (FAT) domain, a four-helix bundle bearing resemblance to similar domains found in Cas family members and vinculin [74]. The FAT contains binding sites for the integrin ligands paxillin [75] and talin [46], as well as for p190Rho-GEF [76]. The C-termini of FAK and its homologue Pyk2 are expressed as separate splice isoforms in certain tissues and have been ascribed inhibitory functions [77, 78].

Activation of FAK in response to integrin activation leads to autophosphorylation of Y397, creating a high-affinity binding site for Src family kinases (SFKs) [79, 80]. FAK and Src can in turn phosphorylate the linker protein p130 Cas, which binds via its SH3 domain to a proline-rich region in the C-terminal half of FAK [81, 82]. A second proline-rich site is bound by the Rho-GAP GRAF [83]. Other known ligands of FAK include phosphatidylinosi-

tol-3-kinase, phospholipase C-gamma and the adaptor protein Grb7 [84–86]. Additionally, FAK and SFKs can bind to and activate several proteins that regulate the actin cytoskeleton, discussed below.

Deletion of the FAK gene in mice leads to embryonic lethality before day E10.5 [87]; FAK-deficient cells show delayed spreading, increased number of focal adhesions and reduced adhesion turnover [88]. This phenotype can be reverted by transfection of the null cells with FAK, provided that it contains a functional autophosphorylation site; p130 Cas binding also contributes to the phenotype [89]. p130 Cas has emerged as a major transducer of FAK-mediated signalling affecting the regulation of focal adhesion turnover (see below).

Integrin-linked kinase

Integrin-linked kinase (ILK) is a non-receptor serine/threonine kinase with an important adaptor protein function. The protein is composed of three N-terminal ankyrin repeats followed by a linker sequence bearing resemblance to a PH domain. The C-terminus contains the kinase domain and mediates direct ILK binding to β1 and β3 cytoplasmic tails [90, 91]. Many of the known interaction partners of ILK also bind to this region. Parvins, proteins composed of two calponin homology (CH) domains, bind to ILK as well as to paxillin, α-actinin, PIX2 and F-actin. Of the three known parvin isoforms, α-parvin/actopaxin and β-parvin/affixin have been shown to bind to ILK [92, 93]. In addition, paxillin binds directly to ILK, and the paxillin binding site of ILK is necessary but not sufficient for targeting ILK to focal adhesions [94]. The first ankyrin repeat of ILK binds Pinch-1 (particularly interesting new Cys-His rich protein) and its homologue Pinch-2 [95–97]. Both Pinch isoforms contain five LIM domains; the first of these mediates binding to ILK [95, 98] and the fourth to the SH2/SH3 adaptor protein Nck2 [99]. ILKAP, a serine/threonine phosphatase, also binds to the N-terminus of ILK [100].

ILK-dependent phosphorylation is debated as a regulatory mechanism for PKB/Akt and GSK3β function [101]. Matrix adhesion and cytoskeletal proteins phosphorylated by ILK include ILK itself [102], β-parvin [93] and β-integrin cytoplasmic tails [90, 91] (reviewed in [103]). Cooperativity between components of multiprotein complexes is a salient feature of ILK-mediated regulation of the actin cytoskeleton. It is known that ILK-PINCH-parvin complex formation protects the individual components from proteosomal degradation, and is essential for the localization of ILK, PINCH and parvin to matrix adhesions [96, 104]. A study addressing the kinetics of ILK-PINCH-parvin recruitment to matrix adhesions has shown that β-parvin and ILK are recruited to early membrane protrusions in CHO cells spreading on fibronectin, as well as the leading edge of lamellipodia and along stress fibres [93]. Additionally, Yamaji and co-workers recently reported that β-parvin binds to α-actinin, which localizes similarly to the leading edge as well as to matrix adhesions and stress fibres [105]. This interaction depends on ILK-dependent β-parvin phosphorylation. Quantitative studies on focal adhesion turnover in cells deficient for components of the ILK-PINCH-parvin complex are required to further elucidate how each individual component contributes to the properties of the complex.

Knockout studies have revealed decisive roles for ILK and PINCH in matrix adhesion formation and turnover, and in actin organization. Deletion of the *ilk* gene in mice leads to peri-implanation lethality, and ILK-deficient fibroblasts show severely impaired adhesion to fibronectin, vitronectin and laminin [106]. The formation of paxillin/vinculin-positive matrix adhesions and of stress fibres is extremely delayed, leading to impaired spreading. This phenotype can be compensated by expression of a mutant of ILK lacking kinase activity. Conditional ablation of ILK in chondrocytes results in reduced adhesion to collagen I and fibronectin and in fewer, more peripherally localized matrix adhesions [107, 108]. Likewise, PINCH-deficient fibroblasts show marked adhesion and migration spreading defects [F. Stanchi and R. F., unpublished].

Paxillin

Paxillin is a modular adaptor protein with important functions in matrix adhesion assembly, turnover and signalling. It is an evolutionarily conserved protein with isoforms present in *D. melanogaster* [109] and *C. elegans* [110]. Paxillin contains five N-terminal LD repeats of 13 amino acids each and 4 C-terminal LIM domains that serve as sites for protein-protein interactions. There are three splice isoforms in mammals of which only paxillin α shows broad expression [111]. Paxillin coimmunoprecipitates with β1 integrin and can bind synthetic peptides mimicking β1 cytoplasmic tails [70, 112], but it binds the α4 cytoplasmic tail with much higher affinity [113]. Paxillin's non-integrin matrix adhesion binding partners all appear to bind through a consensus paxillin binding sequence to one or several LD repeats. Two F-actin binding proteins bind to paxillin, vinculin to LD1, LD2 and LD4 [114], and α-parvin to LD1 and LD4 [115]. Paxillin also binds to the integrin-binding kinases FAK, via LD2 and LD4 [115], and ILK, via LD1 [94], and with the p21-activated kinase isoform PAK3 [116]. Other interactors include the Arf-GAP Git1 [117] and the modular protein paxillin-kinase linker (PKL) [118]. Both of these proteins link paxillin to the Rac1/Cdc42-specific GEF β-PIX/Cool-1. Additionally, Foletta and co-workers recently showed that paxillin coimmunoprecipitates with LIM kinase 1, a regulator of ADF/cofilin [119].

Paxillin can be phosphorylated on four N-terminal tyrosines [120], and there is genetic evidence that the responsible kinases may be FAK [121] and Src family kinases [122]. Tyrosine phosphorylation creates high-affinity binding sites for the SH2 domains of the adaptor protein Crk and for Src [70].

Deletion of the paxillin gene in mice leads to death at E9.5 [123]. The protein appears to be one of the first to be recruited to nascent focal complexes, and its incorporation into $\alpha v\beta 3$-containing focal complexes appears to occur simultaneously with talin [66]. Laukaitis and co-workers have reported that paxillin recruitment is detectable in focal complexes before integrin $\alpha 5\beta 1$ [124]. It is not clear, though, how paxillin is recruited into nascent focal complexes, although a C-terminal focal adhesion targeting sequence appears to be required [114]. The function or binding partners of this sequence, and of all the paxillin LIM domains, are thus far unidentified.

The complex of FAK, Cas and Src with paxillin is emerging as a molecular timer that regulates focal complex turnover. FAK–/– - and paxillin–/– cells display a similar phenotype of reduced migration velocity and slow turnover of matrix adhesions [88, 123]. A systematic study has recently addressed matrix adhesion turnover in mouse embryonic fibroblasts (MEFs) null for Fak, Cas and Src [125]. While paxillin localization to focal adhesions does not require FAK, activated Y397-phosphorylated FAK and tyrosine-phosphorylated paxillin localize to dynamic matrix adhesions and are both required for high turnover rates. In the absence of either paxillin, FAK, Src or Cas, matrix adhesion turnover rates are reduced more than tenfold. Expression of constitutively active Rac or dominant-negative Rho in FAK–/– MEFs does not rescue this defect, arguing against a decisive role of Rho GTPases in the regulation of matrix adhesion turnover via this pathway. A possible mechanism may involve the recruitment of the protease calpain 2 by a Src/FAK/p42ERK complex [126, 127]. Calpain 2 cleaves several matrix adhesion components, including FAK, paxillin, tensin and talin, and calpain-2 knockdown in MEFs results in increased protrusive activity coupled to decreased protrusion persistence [128].

α-Actinin

α-Actinins are proteins originally discovered in skeletal muscle extracts based on their ability to bind and crosslink F-actin. In mammals there are four isoforms encoded by separate genes. The non-muscle α-actinin-1 binds to cytoplasmic tails of $\beta 1$, $\beta 2$ and $\beta 3$ integrins [129–131]. α-Actinin is a modular protein composed of an N-terminal actin-binding domain and a C-terminal EF hand. The interaction between α-actinin and actin is negatively regulated in vitro by FAK-mediated tyrosine phosphorylation on Y12 in the α-actinin actin binding domain [132]. The protein homodimerizes in an antiparallel fashion, allowing for its F-actin crosslinking capacity. α-Actinin has a considerable number of additional binding partners (reviewed in [133]), including the actin-binding proteins vinculin [134] and zyxin [135] as well as the p85 subunit of phosphatidylinositol-3-kinase (PI3K) [136]. Recently, α-actinin-1 has also been shown to bind to β-parvin [105]. α-Actinin mouse knockouts have so far not been reported. 3T3 cells overexpressing α-actinin demonstrate decreased locomotion; conversely, locomotion is increased upon reduction of α-actinin expression by antisense RNA [137]. Chromophore-assisted laser inactivation of α-actinin-GFP fusion protein has demonstrated that α-actinin links actin stress fibres to focal adhesions [138].

Filamin

Filamins are a small family of large (280 kDa) F-actin binding and crosslinking proteins (reviewed in [139]). The non-muscle filamins A and B also bind to β-integrin cytoplasmic tails. The interaction is strong with $\beta 7$ and weaker with $\beta 1$A [44]. The actin-binding domain is located in the N-terminal region, while the remainder of the molecule comprises 24 immunoglobulin (Ig)-like repeats, separated by two hinge regions. These domains mediate protein-protein interactions with other binding partners, and the most C-terminal repeat mediates filamin homo- and heterodimerization [140]. Apart from integrins, filamins associate with other receptors and with many molecules affecting the actin cytoskeleton. These include the small GTPases RhoA, Rac1 and Cdc42 [141], Trio, a GEF for Rac1 and RhoA [142], Rho-associated kinase [143] and a LIM domain-containing protein, Migfilin, which in turn can bind Mig-2 that associates both with ILK and the $\beta 1$-integrin cytoplasmic tail [144]. Filamin A expression can be induced by applying mechanical force to cultured cells [145]. Like α-actinin, filamin-integrin binding appears to negatively regulate cell migration, since CHO cells expressing integrin $\beta 7$ tail fused to the $\beta 3$ extracellular domain showed decreased migration when compared to constructs that were less competent to bind filamin [146]. In man, loss-of-function mutations of the X-linked filamin A gene causes periventricular heterotopia, a hereditary disease characterized by defective neuron migration into the cerebral cortex [147]. The disease leads to embryonic lethality in most male patients. Mouse knockouts of filamin genes have so far not been reported.

Tensin

Tensin is a 220-kDa F-actin binding protein (reviewed in [148]). F-actin binding occurs through three actin binding

domains. Tensin binds to integrins β3, β5 and β7 and more weakly to β1 cytoplasmic tails [149]. A C-terminal SH2 domain can mediate binding to FAK, p130 Cas and PI3K [150, 151]. Tensin-null mice are viable but display skeletal muscle and kidney defects [152, 153]. Tensin-deficient fibroblasts display reduced transwell migration rates, while overexpression of GFP-tensin has the opposite effect [154].

Other proteins

Src family kinases have recently been shown to bind directly to β-integrin cytoplasmic tails, adding another dimension to their important role in integrin-mediated signalling to the actin cytoskeleton [155]. Clustering of integrin β3 results in activation of c-Src by stimulating Src trans autophosphorylation.

RACK1 is an adaptor protein with high similarity to a small G protein β subunit originally described as an effector of various protein kinase C (PKC) isoforms (reviewed in [156]). RACK1 is reported to bind several β cytoplasmic tails [157]. It also binds to and inhibits Src family kinases [158]. Knockdown studies show that loss of RACK1 leads to impaired cell migration, perhaps through a failure to recruit PKCε to focal adhesions [157]. Similar results have been obtained with a RACK1 mutant deficient in Src binding [159].

The unconventional myosin myosin-X has been described recently as an integrin ligand [160]. Myosin-X binds to cytoplasmic tails of integrin β1, β3 and β5 through a C-terminal FERM homology domain. It has been implicated in active transport of integrins to filopodial tips.

The machinery of actin-based motility

The molecular machinery of actin-based motility is highly conserved throughout evolution, and arose before the branching of metazoans from fungi. Actin and many key actin-binding proteins are already present in *Saccharomyces cerevisiae*, where they function in organizing the cortical cytoskeleton and actin cables. A good deal of our current knowledge about the molecules and mechanisms of actin-based cell motility stems from research using unicellular organisms such as *Dictyostelium discoideum* and *Acanthamoeba castellanii*, which lack integrins and matrix adhesions. (*D. discoideum* does have adhesion receptors, which are members of the nine-transmembrane superfamily [161, 162]).

Cell motility requires generation of directed mechanical forces and control of cell body adhesion and tension. Actin filaments are the major building blocks of the membrane protrusions, and the system of matrix adhesions and connecting stress fibres mediate motility. Actin can be present in concentrations close to one millimolar in these structures [163]. In membrane protrusions such as the lamellipodia and filopodia of migrating cells, a considerable forward thrust of some nN/μm^2 [164, 165] is generated by directional treadmilling of a dense, branched actin network against the cell membrane. The key molecule in the generation of this network is the seven-polypeptide Arp2/3 complex [166]. Arp2/3 has the unique activity of inducing autocatalytic branching of actin filaments [167, 168]. A limited set of additional actin-binding proteins has been shown to be sufficient to reconstitute this aspect of actin-based motility in vitro, namely ADF/cofilin, profilin and a barbed-end capping protein such as CapG or gelsolin [169]. Arp2/3 and capping factors act in an antagonistic fashion by balancing barbed end production [168, 170], while ADF/cofilin and profilin can act in synergy with barbed end capping proteins to enhance actin treadmilling velocity [171].

Actin nucleation in the cytoplasm is tightly controlled. The isolated Arp2/3 complex has no endogenous actin-nucleating activity and must be activated by complex formation with activator proteins. The most extensively characterized Arp2/3 activator family are the WASp/Scar proteins, which all bind and activate Arp2/3 through a common C-terminal verprofilin-cofilin-acidic (VCA) domain [172, 173]. The family falls into two subfamilies with N-WASp and its haematopoietic isoform WASp on the one side and the three WAVE/Scar proteins 1, 2 and 3 on the other. All are modular proteins containing binding sites in their N-terminal domains for a variety of cofactors. Purified N-WASp and WASp are constitutively inactive due to intramolecular binding of the regulatory N-terminus to the catalytic VCA-domain [174, 175]. This autoinhibition is relieved through binding of both PIP_2 and the Rho family GTPase Cdc42 [174, 175]. In contrast, WAVE proteins are constitutively active and bind neither Rho family GTPases nor PIP2 directly. Instead, WAVEs assemble into multimolecular complexes together with Nck-associated protein 1 (Nap1) [176], the Rac1-binding protein Sra-1/PIR121 and Abl-interacting protein 1 (Abi1) [177]. The localization of these complexes is regulated by the Rho family GTPase Rac1 [178]. Both N-WASp and the WAVE/Scar isoforms appear to have overlapping but non-identical roles in cell motility, and it is an accepted simplification to describe N-WASp as the main Arp2/3 activator in filopodia [179], while WAVE2 is the predominant Arp2/3 activator in lamellipodia [180]. The central in vivo role of these two family members is underscored by the finding that the disruption of either gene is embryonic lethal in mice [180, 181].

Stiffness of the cell body and retraction of the cell rear are regulated through a mechanism relying not on directed polymerization but on directed or isometric force generation by actomyosin cables (stress fibres) connected to matrix adhesions. The accumulation of these structures as well as the activation of myosin activity is under control

of the RhoA GTPase. RhoA controls stress fibre formation via its effectors Rho-associated kinase (ROCK) and diaphanous-related formins [182].

Integrin effectors meet actin effectors

Integrin signalling has been linked to direct recruitment or activation of various activators of the Arp2/3 complex, thus complementing or perhaps even bypassing GTPase-mediated control mechanisms. Tyrosine phosphorylation of WASp and N-WASp at the residues Y291 and Y256, respectively, increases their potential to activate Arp2/3-mediated actin nucleation [183, 184]. These tyrosine residues lie within the GBD domain of WASp and N-WASp and can be phosphorylated by various Src family kinases. Thus, SFK recruitment by FAK or other members of the matrix adhesion protein complex may provide a docking site for WASp or N-WASp within matrix adhesions and account for the described requirement of WASp/N-WASp for podosome formation in macrophages and v-Src transformed cells, respectively [185, 186]. Additionally, direct interaction between N-WASp and FAK and tyrosine phosphorylation of N-WASp by FAK at Y256 has been reported recently [187]. An alternative mode for targeting N-WASp to matrix adhesions involves recruitment of N-WASp through the adaptor proteins Nck-2 and PINCH [188]. Cortactin is another example for a Src substrate that directly binds and activates the Arp2/3 complex [189]. Cortactin is considered as one of the characteristic protein components of podosomes [38].

Arp2/3 can also be localized to matrix adhesions via binding to vinculin [190]. Although Arp2/3 is not activated upon binding vinculin, vinculin binding may serve to concentrate the complex at the membrane. Individual Arp2/3 subunits may also be regulated through integrin-binding proteins. The regulatory p41-Arc subunit of Arp2/3 was recently reported to be phosphorylated by PAK1, a paxillin ligand, and this phosphorylation is important for promoting cell motility [191].

Regulation of integrin-actin signalling by small molecular weight GTPases

The regulation of Rho family GTPases by matrix adhesion components is certainly an important axis of signalling. The global control of the actin cytoskeleton by the combined action of Rho family GTPases is universal in metazoans [192]. It is subject to control by specialized effector proteins. The transition between GDP-bound inactive Rho and GTP-bound active Rho, and subsequent GTP hydrolysis is controlled by guanine nucleotide exchange factors (GEFs) and GTPase-activating proteins (GAPs), respectively. GEF/GAP activation by phosphorylation and GEF/GAP recruitment to matrix adhesions are important modes of integrin receptor signalling to the actin cytoskeleton.

Pioneering studies have demonstrated a global role for integrin-mediated adhesion in the regulation of small GTPase activity. Within the first 10 min following substrate adhesion, fibroblast spreading on fibronectin is accompanied by an initial increase in Rac and Cdc42 activity, as assayed by an increase in PAK kinase activity [193]. This is followed by an increase in Rho GTP loading [194]. β-Integrin tails play an important role in Rac activation since mutation of the β1 tail leads to impaired Rac signalling [195], and clustering of β tails in human primary fibroblasts is both necessary and sufficient for Rac activation [196].

So far Eps8, which forms part of a RacGEF complex with Abi-1 and Sos-1 [197], is the only GEF identified to target to matrix adhesions via a direct interaction with the β1A cytoplasmic tail [149]. Instead, many GEFs are localized to matrix adhesions indirectly via binding to other integrin cytoplasmic ligands, perhaps reflecting the evolution of integrin control over this ancient system. Only some of the involved integrin-GEF-GTPase pathways have so far been studied in detail. Rac/Cdc42 activation appears to occur through several parallel pathways. Activated FAK can recruit the non-conventional Rac-GEF DOCK180, the SH2/SH3 adaptor protein Crk and p130Cas [198]. Paxillin can recruit the Rac/Cdc42-GEF β-PIX/Cool-1 in complex with PAK and PKL [199] or with Git1 [117]. Coimmunoprecipitation and GST pull-down assays have revealed that β-parvin binds to the related GEF α-PIX/Cool-2 [200]. Trio, which possesses GEF activity both for Rac and RhoA, binds to and colocalizes with filamin [142]. The preponderance of GEFs found localized to matrix adhesions suggests that trafficking of actin cytoskeleton-regulating GTPases to matrix adhesions results in activation of these GTPases at sites of membrane-ECM attachment.

Integrin engagement also leads to translocation of inactive Rac and Cdc42 from the cytoplasm to the membrane and their activation by dissociating them from RhoGDI [201]. The authors have recently shown that Rac1 is targeted preferentially to lipid rafts and that integrin-mediated adhesion prevents the rapid internalization of lipid rafts that occurs when cells are in suspension [202].

The transient RhoA suppression associated with cell spreading has been ascribed to p190RhoGAP, which is activated upon early integrin signalling in a Src-dependent manner [203]. The current hypothesis to explain how this occurs states that p190RhoGAP is liberated from a complex with p120RasGAP upon recruitment of p120RasGAP to paxillin phosphorylated by FAK/Src on Y31/Y118 [204]. How RhoA activation is mediated is less clear. FAK binds to p190RhoGEF; overexpression of

FAK leads to increased p190RhoGEF phosphorylation and increased RhoA activity [76]. Rho targeting to the leading edge of cells is, like Rac, mediated by lipid rafts. Palazzo and co-workers have shown that lipid rafts are localized to the leading edge by an unknown mechanism that requires FAK, and that these lipid rafts are important for local coupling of Rho to mDia and that integrins localize such lipid rafts [205].

Regulation of integrin-actin signalling by lipid kinases

Polyphosphoinositides (PPIs) have emerged as important regulators of focal adhesion and actin dynamics, particularly the PPIs PI(4,5)P_2, synthesized by phosphatidylinositol phosphate (PIP) kinases, and PI(3,4,5)P_3, synthesized by PI-3 kinases. As more becomes known about the biochemical roles of these PPIs, it appears as though PI(4,5)P_2 plays a role in mediating both cell adhesion and migration, whereas PI(3,4,5)P_3 plays a greater role in mediating cell migration.

Expression of constitutively active PI-3 kinase in cells leads to an increased level of activated Rac and increased lamellipodia and ruffle formation [206]. Conversely, treatment of several cell types with the PI-3 kinase inhibitor wortmannin results in decreased amounts of activated Rac and Cdc42 [207–209]. Although Rac and PI-3 kinase directly interact [210] and activated Rac and Cdc42 increase PI-3 kinase activity [211], PI(3,4,5)P_3 does not appear to influence these GTPases directly. Rather, several GEFs contain PH domains that bind PI(3,4,5)P_3 (reviewed in [212]). Binding of PI(3,4,5)P_3 to the PH domain is thought to induce a conformational change in these GEFs, thereby relieving autoinhibitory mechanisms.

The migration of chemotactic cells up a shallow chemical gradient is regulated by establishing a relatively steep intracellular gradient of PI(3,4,5)P_3, by localizing PI-3 kinase at the leading edge and the phosphatase PTEN towards the rear of the cell [213]. Activation of PI-3 kinase under chemotactic conditions is dependent upon heterotrimeric G proteins which sense extracellular chemical gradients (reviewed in [214]). Integrin-mediated migration, on the other hand, depends on PI-3 kinase present within the matrix adhesion. PI-3 kinase binds via its 85-kDa regulatory subunit to a sequence including phosphotyrosine Y397 within FAK [84]. Mutation of D395 greatly diminishes PI-3 kinase binding to FAK, resulting in an inhibition of cell migration to an even greater extent than is achieved by treating cells with wortmannin or another PI-3 kinase inhibitor, LY294002 [215]. The strength of adhesion to the substrate was unaffected by these drugs, demonstrating that PI(3,4,5)P_3 is not important for anchoring cells to the substratum.

Recently another product of PI-3 kinase, PI(3,4)P_2, was shown to bind lamellipodin, an Ena/VASP ligand present at the edges of lamellipodia [216]. Treatment of cells with PDGF, which causes an increase in PI(3,4)P_2 synthesis [217], is sufficient to target a lamellipodin PH domain green fluorescent protein (GFP) fusion protein to the tips of lamellipodia, implicating PI(3,4)P_2 binding in correct targeting of lamellipodin to lamellipodial tips, and therefore Ena/VASP proteins to the leading edge of migrating cells.

Although the effects of PI(3,4,5)P_3 on influencing the architecture of matrix adhesions appear limited to α-actinin [218], there are several lines of evidence implicating PI(4,5)P_2 in mediating protein-protein interactions between several matrix adhesion binding proteins. PI(4,5)P_2 binding has been demonstrated for talin [48], filamin [219], vinculin and α-actinin [220]. PI(4,5)P_2 binding to talin causes the localization of talin to matrix adhesions, presumably by increasing the affinity between talin and the cytoplasmic tail of β1 integrin [48]. Vinculin binding to PI(4,5)P_2 induces a conformational change in vinculin, unmasking domains responsible for binding talin and actin [64, 221]. However, actin binding is inhibited by PI(4,5)P_2, suggesting that there are other regulatory steps downstream of PI(4,5)P_2 binding [222]. Filamin is inhibited in the presence of PI(4,5)P_2 [219], while α-actinin actin cross-linking activity is either enhanced [223] or reduced [224] in the presence of PI(4,5)P_2; the reason for the discrepancy is not known.

It is well established that adhesion of cells to ECM causes a transient increase in PI(4,5)P_2 concentration, and that at least some of this PI(4,5)P_2 is localized at matrix adhesions [225]. Local production of PI(4,5)P_2 at sites of matrix adhesions likely arises from the γ isoform of type I phosphatidylinositol phosphate kinase (PIPKIγ). PIPKIγ targets to focal adhesions by binding to the FERM domain of talin [48, 49]. Tyrosine phosphorylation of the talin-binding sequence of PIPKIγ by Src increases the affinity of PIPKIγ for talin, in a FAK-dependent manner [49, 226]. It was also shown that the activity of PIPKIγ increases upon tyrosine phosphorylation, but this may also arise from controlling the phosphorylation status of a serine residue (S214) which regulates the catalytic activity of PIPKIα [227, 228]. Furthermore, the PIPKIγ binding site on talin overlaps with the β1-integrin binding site, and binding of PIPKIγ and β1 integrin are mutually exclusive [226, 229]. Therefore, PIPKIγ may also serve to regulate the turnover of matrix adhesions by blocking the interaction between talin and β1 integrin.

On the actin side, WASP family proteins, cofilin, profilin, gelsolin and CapZ have all been shown to be regulated by PI(4,5)P_2 (see [230] and references therein). The control of PI(4,5)P_2 synthesis for this purpose may be achieved by Rho family GTPases. PIPKI activity is present in Rac1 immunoprecipitates, and a ternary complex between Rac1, RhoGDI and PIPKI has been identified [231, 232]. Furthermore, activation of PIPKI activity in vivo is en-

hanced by expression of RhoA, Rac1 and Cdc42 [233]; the RhoA-dependent activation is mediated by Rho-kinase [234, 235], but it is not known whether Rac1 and Cdc42 can activate PIPKI directly, or whether other proteins are responsible. In all cases, it is likely that activation of PIPKI occurs once it has been targeted to the plasma membrane [236]. In this way, $PI(4,5)P_2$ availability for actin binding proteins may be spatio-temporally controlled by Rho family GTPases.

Differential integrin signalling to the cytoskeleton

Distinct in vivo roles for different integrin receptors have been amply demonstrated by gene ablation studies (reviewed in [237]). Considering that many of the proteins discussed above can bind to several different β cytoplasmic tails, how does signalling downstream of $\alpha3\beta1$ differ from $\alpha5\beta1$, or $\alpha V\beta3$? First, signals may be directly transmitted by the extracellular and transmembrane domains of integrin receptors. Second, although most known cytoplasmic integrin ligands bind to β tails, there are examples of differential signalling through α tail ligands. Third, some protein ligands are highly specific for only one β isoform or splice variant. Fourth, integrin β tails are posttranslationally modified by phosphorylation and proteolytic cleavage that affect ligand binding, and many β tail binding proteins have homologues or splice isoforms expressed in a cell type-specific fashion.

Table 1 proposes a classification of the human integrin receptors by the probable evolutionary relationship of the β chains [3, 5]. This subdivision yields three groups comprising $\beta1$, $\beta2$ and $\beta7$; $\beta3$, $\beta5$, $\beta6$ and $\beta8$; and $\beta4$. The $\beta1$ group alone comprises two thirds of all integrin heterodimers. It comes as no surprise that $\beta1$-integrin gene disruption shows the most severe phenotype of all β chains, leading to peri-implantation lethality [238, 239]. The $\beta2$ and $\beta7$ subgroups are hematopoietic cell-specific. Four $\beta1$ integrins ($\alpha1\beta1$, $\alpha2\beta1$, $\alpha10\beta1$ and $\alpha11\beta1$) form the collagen receptor subfamily [240], present only in chordates. The members of the $\beta3$ subfamily are all RGD receptors formed by association with αV, with the notable exception of αIIb$\beta3$, the major integrin receptor in platelets. $\beta8$ appears to have diverged early from the rest of the group since the cytoplasmic domain shows low homology with $\beta3$, $\beta5$ and $\beta6$, and it lacks the otherwise conserved NPxY motifs. $\beta3$-deficient mice are viable and fertile and show normal retinal neovascularization, but they display bleeding disorders related to αIIb$\beta3$ and increased tumor angiogenesis [241, 242]. In contrast, antibodies against $\alpha v\beta3$ integrin, intended to block the function of this integrin, inhibit angiogenesis [241–243]. Possible reasons for this discrepancy have been discussed [244–246]. $\beta4$ associates exclusively with $\alpha6$ and defines a unique member of the integrin β chain family by virtue of the size of its cytoplasmic domain (>1000 aa). It is well established that $\alpha6\beta4$ is a laminin receptor which also binds to intermediate filaments and is a key component of hemidesmosomes in epithelial cells. Mechanisms of $\beta4$-mediated signalling in adhesion and motility are currently under investigation (reviewed in [247]) but so far do not appear to involve the integrin-binding ligands discussed above. We will therefore limit the following discussion to the $\beta1$ and $\beta3$ groups.

Differential regulation of cell adhesion and small GTPase activity by fibronectin receptors has been demonstrated by ectopic expression of $\beta1$ or $\beta3$ in $\beta1$-deficient fibroblasts [248]. While Rac1 GTP loading was equally high in both cell lines, RhoA signalling was markedly different. Both cell lines showed decreased RhoA activity immediately following binding to fibronectin, but only cells expressing $\beta1$ integrin subsequently displayed increased RhoA GTP loading. Cells expressing only $\beta3$ integrin showed high RhoA GTP loading in suspension but no subsequent increase upon binding to fibronectin. Tyrosine phosphorylation of p190RhoGAP was comparable in both lines, arguing against differential regulation of RhoA activity through phosphorylation of 190RhoGAP by FAK and/or Src. In a similar study, Miao and co-authors investigated the effects of $\beta1$ and $\beta3$ overexpression in CHO cells, which do not express endogenous $\beta3$ integrin [249]. Rac activity was higher in $\beta1$-expressing cells while only $\beta3$-expressing cells showed an increase in RhoA GTP loading during late spreading. Both groups also used mutation constructs in which the extracellular domain of $\beta1$ was fused to the $\beta3$ cytoplasmic tail or the extracellular domain of $\beta1$ was mutated to acquire $\beta3$ specificity, respectively. Strikingly, cells expressing these constructs gave results similar to $\beta1$ or $\beta3$ expression, respectively. These results demonstrate that the extracellular domain of β subunits can play a decisive role in integrin signalling specificity, possibly through integrin-integrin or integrin-RTK crosstalk.

Paxillin binding to $\alpha4$ integrin is arguably the best-characterized example for differential signalling through an α-integrin cytoplasmic tail. $\alpha4\beta1$ differs from other RGD-binding integrin receptors in that it enhances the velocity and persistence of cell migration when expressed endogenously or ectopically [250]. $\alpha4$ binds tightly to paxillin [113]; this interaction is disrupted by PKA-mediated phosphorylation of $\alpha4$ on S988 [251, 252]. In CHO cells transfected with wild-type $\alpha4$, phospho-$\alpha4$ localizes to the leading edge, while the non-phosphorylated, paxillin-bound $\alpha4$ localizes to the lateral and trailing edges. Expression of a S998A mutant or of an $\alpha4$-paxillin chimera results in impaired migration.

Both $\beta1$- and $\beta3$-integrin genes can undergo alternative splicing to generate different C-termini adjacent to the membrane-proximal part of the cytoplasmic domain, termed $\beta1$A-D and $\beta3$A-C. The ubiquitous $\beta1$A and the

muscle-specific β1D variants share high homology and localize to focal adhesions when expressed in non-muscle cells [253, 254]. In contrast, β1B and β1C, which are human-specific, are deficient in binding to a number of ligands, including talin [44]. Consistent with a possible inhibitory role of some of these splice variants in malignant cell progression, Moro and co-workers have recently detected that expression of β1C is posttransscriptionally downregulated in a prostate carcinoma cell line, when compared to a non-cancerous control cell line [255].

Tyrosine phosphorylation of β tails by SFKs appears to be relevant for a number of physiological processes. Inducible tyrosine phosphorylation of the β3 cytoplasmic tail on Y747 has been implicated in regulating the avidity of αvβ3 [256, 257]. Tyrosine phosphorylation of the β3 tail is also important for normal platelet function in vivo as demonstrated by the expression of β3 tails in which this residue was mutated [258] as well as the observation that binding of the adaptor proteins Grb2 and Shc to β3 cytoplasmic tails is dependent on tyrosine phosphorylation of the cytoplasmic tail [259]. The recently reported recruitment of Arp2/3 into matrix adhesions is also dependent on phosphorylation of β3 on Y747 [260].

Serine/threonine phosphorylation of β2-integrin cytoplasmic tails correlates with increased association with cytoskeletal cell fractions [261]. PKCδ and PKCβ were shown to be the main kinases in leukocyte extracts that phosphorylate β2 peptides [262].

Proteolytic cleavage of integrin cytoplasmic tails may provide another mechanism to regulate ligand binding to integrin cytoplasmic tails. Calpain cleavage was first demonstrated for the β3 cytoplasmic domain in vivo [263]; subsequently, calpain cleavage sites were identified in several other β tails [264]. Several components of focal adhesions including talin, FAK, paxillin, vinculin and α-actinin are also cleaved by calpain in vivo [54, 128, 265]; therefore protease cleavage likely plays an important role in regulating matrix adhesion turnover. Differential cleavage of β tails could also be implicated in regulating integrin signalling.

Finally, isoforms of β-integrin tail binding proteins (summarized in table 2) are likely to be involved in differential regulation of integrin-actin signalling by cell type-specific expression and unique binding properties. For example, the FAK isoform Pyk2 (alternatively named CAKβ, RAFTK or CADTK), which is highly expressed in brain and hematopoietic cells [266], appears to have additional or even antagonistic functions in comparison to FAK. A recent study [267] reported positive and negative correlation between Pyk2 and FAK expression, respectively, with cell migration rates in glioblastoma cell lines. Cells that do not express FAK but express Pyk2 also express the paxillin isoform leupaxin. It has been suggested that Pyk2 and leupaxin may form a hematopoetic cell-specific signalling complex [268]. An impressive illustration of the tissue specificity of integrin-mediated signalling in mammals is given by the phenotype of mice with a targeted deletion of the α8 subunit. 50% of these α8–/– mice are born without kidneys, but without major defects in other inner organs [269].

Conclusions and perspectives

We dispose today of a solid base to qualitatively explain many aspects of integrin-mediated signalling to the actin cytoskeleton. However, our current framework of model pathways is still very preliminary. While new downstream effectors and crosstalk partners of integrin signalling are constantly being discovered, there is still scarce data on the affinities and especially on the topology of binding of the identified β tail ligands to β tails and among each other. Many of these interactions are likely to be mutually exclusive, while others may be cooperative. To understand which subset(s) of integrin-proximal protein complexes actually mediate integrin-actin signalling in a specific cell type, careful biochemical and structural analyses will have to be combined with experiments on cells or animals expressing mutant forms of matrix adhesion proteins. As in the study of actin-based motility, reconstituted model systems would certainly be of great benefit to gain a quantitative understanding of the molecular mechanisms of integrin-actin signalling. The ‘matrix adhesion on a chip’ is not reality yet, but biophysical methods are increasingly being used to analyze and manipulate matrix adhesions in living cells. Finally, much uncharted territory remains to be explored beyond matrix adhesions. Integrins are localized to many actin-containing structures such as the lamellipodial leading edge, filopodial tips and the actomyosin contractile ring. Their contribution to the proper function of these structures, let alone the implicated signalling pathways, is unknown.

Acknowledgements. This work was supported by a DFG grant (SFB413, S.W.) and a Marie Curie International Fellowship within the 6th European Community Framework Programme to K.R.L.

1 Ridley A. J., Schwartz M. A., Burridge K., Firtel R. A., Ginsberg M. H., Borisy G. et al. (2003) Cell migration: integrating signals from front to back. Science **302:** 1704–1709
2 Friedl P., Brocker E. B. and Zanker K. S. (1998) Integrins, cell matrix interactions and cell migration strategies: fundamental differences in leukocytes and tumor cells. Cell Adhes. Commun. **6:** 225–236
3 Burke R. D. (1999) Invertebrate integrins: structure, function and evolution. Int. Rev. Cytol. **191:** 257–284
4 Hynes R. O. and Zhao Q. (2000) The evolution of cell adhesion. J. Cell Biol. **150:** F89–F96
5 Hughes A. L. (2001) Evolution of the integrin alpha and beta protein families. J. Mol. Evol. **52:** 63–72
6 van der Flier A. and Sonnenberg A. (2001) Function and interactions of integrins. Cell Tissue Res. **305:** 285–298

7 Hynes R. O. (2002) Integrins: bidirectional, allosteric signaling machines. Cell **110:** 673–687
8 Wehrle-Haller B. and Imhof B. A. (2003) Integrin-dependent pathologies. J. Pathol. **200:** 481–487
9 Kalluri R. (2003) Basement membranes: structure, assembly and role in tumour angiogenesis. Nat. Rev. Cancer **3:** 422–433
10 Kerr J. R. (1999) Cell adhesion molecules in the pathogenesis of and host defence against microbial infection. Mol. Pathol. **52:** 220–230
11 Liddington R. C. and Ginsberg M. H. (2002) Integrin activation takes shape. J. Cell Biol. **158:** 833–839
12 Calderwood D. A. (2004) Talin controls integrin activation. Biochem. Soc. Trans. **32:** 434–437
13 Xiong J. P., Stehle T., Zhang R., Joachimiak A., Frech M., Goodman S. L. et al. (2002) Crystal structure of the extracellular segment of integrin alpha Vbeta3 in complex with an Arg-Gly-Asp ligand. Science **296:** 151–155
14 Xiao T., Takagi J., Coller B. S., Wang J. H. and Springer T. A. (2004) Structural basis for allostery in integrins and binding to fibrinogen-mimetic therapeutics. Nature **432:** 59–67
15 Takagi J., Strokovich K., Springer T. A. and Walz T. (2003) Structure of integrin alpha5beta1 in complex with fibronectin. EMBO J. **22:** 4607–4615
16 Bellis S. L. (2004) Variant glycosylation: an underappreciated regulatory mechanism for beta1 integrins. Biochim. Biophys. Acta **1663:** 52–60
17 Williams B. D. and Waterston R. H. (1994) Genes critical for muscle development and function in *Caenorhabditis elegans* identified through lethal mutations. J. Cell Biol. **124:** 475–490
18 Yamada K. M. and Even-Ram S. (2002) Integrin regulation of growth factor receptors. Nat. Cell Biol. **4:** E75–E76
19 Lee J. W. and Juliano R. (2004) Mitogenic signal transduction by integrin- and growth factor receptor-mediated pathways. Mol. Cells **17:** 188–202
20 Wang R., Kobayashi R. and Bishop J. M. (1996) Cellular adherence elicits ligand-independent activation of the Met cell-surface receptor. Proc. Natl. Acad. Sci. USA **93:** 8425–8430
21 Sundberg C. and Rubin K. (1996) Stimulation of beta1 integrins on fibroblasts induces PDGF independent tyrosine phosphorylation of PDGF beta-receptors. J. Cell Biol. **132:** 741–752
22 Wang J. F., Zhang X. F. and Groopman J. E. (2001) Stimulation of beta 1 integrin induces tyrosine phosphorylation of vascular endothelial growth factor receptor-3 and modulates cell migration. J. Biol. Chem. **276:** 41950–41957
23 Moro L., Venturino M., Bozzo C., Silengo L., Altruda F., Beguinot L. et al (1998) Integrins induce activation of EGF receptor: role in MAP kinase induction and adhesion-dependent cell survival. EMBO J. **17:** 6622–6632
24 Kuwada S. K. and Li X. (2000) Integrin alpha5/beta1 mediates fibronectin-dependent epithelial cell proliferation through epidermal growth factor receptor activation. Mol. Biol. Cell **11:** 2485–2496
25 Schneller M., Vuori K. and Ruoslahti E. (1997) Alphavbeta3 integrin associates with activated insulin and PDGFbeta receptors and potentiates the biological activity of PDGF. EMBO J. **16:** 5600–5607
26 Schlaepfer D. D., Hanks S. K., Hunter T. and van der Geer P. (1994) Integrin-mediated signal transduction linked to Ras pathway by GRB2 binding to focal adhesion kinase. Nature **372:** 786–791
27 Vuori K., Hirai H., Aizawa S. and Ruoslahti E. (1996) Introduction of p130cas signaling complex formation upon integrin-mediated cell adhesion: a role for Src family kinases. Mol. Cell Biol. **16:** 2606–2613
28 Barberis L., Wary K. K., Fiucci G., Liu F., Hirsch E., Brancaccio M. et al. (2000) Distinct roles of the adaptor protein Shc and focal adhesion kinase in integrin signaling to ERK. J. Biol. Chem. **275:** 36532–36540
29 Maeshima Y., Colorado P. C., Torre A., Holthaus K. A., Grunkemeyer J. A., Ericksen M. B. et al. (2000) Distinct antitumor properties of a type IV collagen domain derived from basement membrane. J. Biol. Chem. **275:** 21340–21348
30 Giancotti F. G. and Ruoslahti E. (1990) Elevated levels of the alpha 5 beta 1 fibronectin receptor suppress the transformed phenotype of Chinese hamster ovary cells. Cell **60:** 849–859
31 Stupack D. G., Puente X. S., Boutsaboualoy S., Storgard C. M. and Cheresh D. A. (2001) Apoptosis of adherent cells by recruitment of caspase-8 to unligated integrins. J. Cell Biol. **155:** 459–470
32 Stupack D. G. and Cheresh D. A. (2004) A Bit-role for integrins in apoptosis. Nat. Cell Biol. **6:** 388–389
33 Hood J. D. and Cheresh D. A. (2002) Role of integrins in cell invasion and migration. Nat.Rev. Cancer **2:** 91–100
34 Brakebusch C., Bouvard D., Stanchi F., Sakai T. and Fassler R. (2002) Integrins in invasive growth. J. Clin. Invest **109:** 999–1006
35 Friedl P. and Wolf K. (2003) Tumour-cell invasion and migration: diversity and escape mechanisms. Nat. Rev. Cancer **3:** 362–374
36 Geiger B., Bershadsky A., Pankov R. and Yamada K. M. (2001) Transmembrane crosstalk between the extracellular matrix-cytoskeleton crosstalk. Nat. Rev. Mol. Cell Biol. **2:** 793–805
37 Zamir E., Katz M., Posen Y., Erez N., Yamada K. M., Katz B. Z. et al. (2000) Dynamics and segregation of cell-matrix adhesions in cultured fibroblasts. Nat. Cell Biol. **2:** 191–196
38 Linder S. and Aepfelbacher M. (2003) Podosomes: adhesion hot-spots of invasive cells. **13:** 376–385
39 Tarone G., Cirillo D., Giancotti F. G., Comoglio P. M. and Marchisio P. C. (1985) Rous sarcoma virus-transformed fibroblasts adhere primarily at discrete protrusions of the ventral membrane called podosomes. Exp. Cell Res. **159:** 141–157
40 Spinardi L., Rietdorf J., Nitsch L., Bono M., Tacchetti C., Way M. et al. (2004) A dynamic podosome-like structure of epithelial cells. Exp. Cell Res. **295:** 360–374
41 Nayal A., Webb D. J. and Horwitz A. F. (2004) Talin: an emerging focal point of adhesion dynamics. Curr. Opin. Cell Biol. **16:** 94–98
42 Campbell I. D. and Ginsberg M. H. (2004) The talin-tail interaction places integrin activation on FERM ground. Trends Biochem. Sci. **29:** 429–435
43 Horwitz A., Duggan K., Buck C., Beckerle M. C. and Burridge K. (1986) Interaction of plasma membrane fibronectin receptor with talin – a transmembrane linkage. Nature **320:** 531–533
44 Pfaff M., Liu S., Erle D. J. and Ginsberg M. H. (1998) Integrin beta cytoplasmic domains differentially bind to cytoskeletal proteins. J. Biol. Chem. **273:** 6104–6109
45 Garcia-Alvarez B., de Pereda J. M., Calderwood D. A., Ulmer T. S., Critchley D., Campbell I. D. et al (2003) Structural determinants of integrin recognition by talin. Mol. Cell **11:** 49–58
46 Chen H. C., Appeddu P. A., Parsons J. T., Hildebrand J. D., Schaller M. D. and Guan J. L. (1995) Interaction of focal adhesion kinase with cytoskeletal protein talin. J. Biol. Chem. **270:** 16995–16999
47 Martel V., Racaud-Sultan C., Dupe S., Marie C., Paulhe F., Galmiche A. et al. (2001) Conformation, localization and integrin binding of talin depend on its interaction with phosphoinositides. J. Biol. Chem. **276:** 21217–21227
48 Di Paolo G., Pellegrini L., Letinic K., Cestra G., Zoncu R., Voronov S. et al. (2002) Recruitment and regulation of phosphatidylinositol phosphate kinase type 1 gamma by the FERM domain of talin. Nature **420:** 85–89
49 Ling K., Doughman R. L., Firestone A. J., Bunce M. W. and Anderson R. A. (2002) Type I gamma phosphatidylinositol

phosphate kinase targets and regulates focal adhesions. Nature **420:** 89–93
50 Borowsky M. L. and Hynes R. O. (1998) Layilin, a novel talin-binding transmembrane protein homologous with C-type lectins, is localized in membrane ruffles. J. Cell Biol. **143:** 429–442
51 Xing B., Jedsadayanmata A. and Lam S. C. (2001) Localization of an integrin binding site to the C terminus of talin. J. Biol. Chem. **276:** 44373–44378
52 Hemmings L., Rees D. J., Ohanian V., Bolton S. J., Gilmore A. P., Patel B. et al. (1996) Talin contains three actin-binding sites each of which is adjacent to a vinculin-binding site. J. Cell Sci. **109 (Pt 11):** 2715–2726
53 Muguruma M., Matsumura S. and Fukazawa T. (1990) Direct interactions between talin and actin. Biochem. Biophys. Res. Commun. **171:** 1217–1223
54 Franco S. J., Rodgers M. A., Perrin B. J., Han J., Bennin D. A., Critchley D. R. et al. (2004) Calpain-mediated proteolysis of talin regulates adhesion dynamics. Nat. Cell Biol. **6:** 977–983
55 Nuckolls G. H., Romer L. H. and Burridge K. (1992) Microinjection of antibodies against talin inhibits the spreading and migration of fibroblasts. J. Cell Sci. **102 (Pt 4):** 753–762
56 Monkley S. J., Zhou X. H., Kinston S. J., Giblett S. M., Hemmings L., Priddle H. et al (2000) Disruption of the talin gene arrests mouse development at the gastrulation stage. Dev. Dyn. **219:** 560–574
57 Priddle H., Hemmings L., Monkley S., Woods A., Patel B., Sutton D. et al. (1998) Disruption of the talin gene compromises focal adhesion assembly in undifferentiated but not differentiated embryonic stem cells. J. Cell Biol. **142:** 1121–1133
58 Tadokoro S., Shattil S. J., Eto K., Tai V., Liddington R. C., de Pereda J. M. et al. (2003) Talin binding to integrin beta tails: a final common step in integrin activation. Science **302:** 103–106
59 Calderwood D. A., Zent R., Grant R., Rees D. J., Hynes R. O., and Ginsberg M. H. (1999) The Talin head domain binds to integrin beta subunit cytoplasmic tails and regulates integrin activation. J. Biol. Chem. **274:** 28071–28074
60 Jiang G., Giannone G., Critchley D. R., Fukumoto E. and Sheetz M. P. (2003) Two-piconewton slip bond between fibronectin and the cytoskeleton depends on talin. Nature **424:** 334–337
61 Giannone G., Jiang G., Sutton D. H., Critchley D. R. and Sheetz M. P. (2003) Talin1 is critical for force-dependent reinforcement of initial integrin-cytoskeleton bonds but not tyrosine kinase activation. J. Cell Biol. **163:** 409–419
62 Vinogradova O., Velyvis A., Velyviene A., Hu B., Haas T., Plow E. et al. (2002) A structural mechanism of integrin alpha(IIb)beta(3) 'inside-out' activation as regulated by its cytoplasmic face. Cell **110:** 587–597
63 Kim M., Carman C. V, and Springer T. A. (2003) Bidirectional transmembrane signaling by cytoplasmic domain separation in integrins. Science **301:** 1720–1725
64 Gilmore A. P. and Burridge K. (1996) Regulation of vinculin binding to talin and actin by phosphatidyl-inositol-4-5-bisphosphate. Nature **381:** 531–535
65 Xu W., Coll J. L. and Adamson E. D. (1998) Rescue of the mutant phenotype by reexpression of full-length vinculin in null F9 cells; effects on cell locomotion by domain deleted vinculin. J. Cell Sci. **111 (Pt 11):** 1535–1544
66 Zaidel-Bar R., Ballestrem C., Kam Z. and Geiger B. (2003) Early molecular events in the assembly of matrix adhesions at the leading edge of migrating cells. J. Cell Sci. **116:** 4605–4613
67 Kreitmeier M., Gerisch G., Heizer C. and Muller-Taubenberger A. (1995) A talin homologue of *Dictyostelium* rapidly assembles at the leading edge of cells in response to chemoattractant. J. Cell Biol. **129:** 179–188
68 Hibi M., Nagasaki A., Takahashi M., Yamagishi A. and Uyeda T. Q. (2004) *Dictyostelium discoideum* talin A is crucial for myosin II-independent and adhesion-dependent cytokinesis. J. Muscle Res. Cell Motil.127–140
69 Parsons J. T. (2003) Focal adhesion kinase: the first ten years. J. Cell Sci. **116:** 1409–1416
70 Schaller M. D., Otey C. A., Hildebrand J. D. and Parsons J. T. (1995) Focal adhesion kinase and paxillin bind to peptides mimicking beta integrin cytoplasmic domains. J. Cell Biol. **130:** 1181–1187
71 Shen Y. and Schaller M. D. (1999) Focal adhesion targeting: the critical determinant of FAK regulation and substrate phosphorylation. Mol. Biol. Cell **10:** 2507–2518
72 Cooper L. A., Shen T. L. and Guan J. L. (2003) Regulation of focal adhesion kinase by its amino-terminal domain through an autoinhibitory interaction. Mol. Cell Biol. **23:** 8030–8041
73 Dunty J. M., Gabarra-Niecko V., King M. L., Ceccarelli D. F., Eck M. J. and Schaller M. D. (2004) FERM domain interaction promotes FAK signaling. Mol. Cell Biol. **24:** 5353–5368
74 Arold S. T., Hoellerer M. K. and Noble M. E. (2002) The structural basis of localization and signaling by the focal adhesion targeting domain. Structure (Camb.) **10:** 319–327
75 Hildebrand J. D., Schaller M. D. and Parsons J. T. (1995) Paxillin, a tyrosine phosphorylated focal adhesion-associated protein binds to the carboxyl terminal domain of focal adhesion kinase. Mol. Biol. Cell **6:** 637–647
76 Zhai J., Lin H., Nie Z., Wu J., Canete-Soler R., Schlaepfer W. W. et al. (2003) Direct interaction of focal adhesion kinase with p190RhoGEF. J. Biol. Chem. **278:** 24865–24873
77 Taylor J. M., Mack C. P., Nolan K., Regan C. P., Owens G. K. and Parsons J. T. (2001) Selective expression of an endogenous inhibitor of FAK regulates proliferation and migration of vascular smooth muscle cells. Mol. Cell Biol. **21:** 1565–1572.
78 Xiong W. C., Macklem M. and Parsons J. T. (1998) Expression and characterization of splice variants of PYK2, a focal adhesion kinase-related protein. J. Cell Sci. **111 (Pt 14):** 1981– 1991
79 Cobb B. S., Schaller M. D., Leu T. H. and Parsons J. T. (1994) Stable association of pp60src and pp59fyn with the focal adhesion-associated protein tyrosine kinase, pp125FAK. Mol. Cell Biol. **14:** 147–155
80 Schaller M. D., Hildebrand J. D., Shannon J. D., Fox J. W., Vines R. R. and Parsons J. T. (1994) Autophosphorylation of the focal adhesion kinase, pp125FAK, directs SH2-dependent binding of pp60src. Mol. Cell Biol. **14:** 1680–1688
81 Polte T. R. and Hanks S. K. (1995) Interaction between focal adhesion kinase and Crk-associated tyrosine kinase substrate p130Cas. Proc. Natl. Acad. Sci. USA **92:** 10678–10682
82 O'Neill G. M., Fashena S. J. and Golemis E. A. (2000) Integrin signalling: a new Cas(t) of characters enters the stage. Trends Cell Biol. **10:** 111–119
83 Hildebrand J. D., Taylor G. M. and Parsons J. T. (1996) An SH3 domain-containing GTPase-activating protein for Rho and Cdc42 associates with focal adhesion kinase. Mol. Cell. Biol. **16:** 3169–1378
84 Chen H. C., Appeddu P. A., Isoda H. and Guan J. L. (1996) Phosphorylation of tyrosine 397 in focal adhesion kinase is required for binding phosphatidylinositol 3-kinase. J. Biol. Chem. **271:** 26329–26334
85 Chen H. C. and Guan J. L. (1994) Stimulation of phosphatidylinositol 3′-kinase association with foca adhesion kinase by platelet-derived growth factor. J. Biol. Chem. **269:** 31229–31233
86 Han D. C. and Guan J. L. (1999) Association of focal adhesion kinase with Grb7 and its role in cell migration. J. Biol. Chem. **274:** 24425–24430
87 Furuta Y., Ilic D., Kanazawa S., Takeda N., Yamamoto T. and Aizawa S. (1995) Mesodermal defect in late phase of gastrulation by a targeted mutation of focal adhesion kinase, FAK. Oncogene **11:** 1989–1995

88 Ilic D., Furuta Y., Kanazawa S., Takeda N., Sobue K., Nakatsuji N. et al. (1995) Reduced cell motility and enhanced focal adhesion contact formation in cells from FAK-deficient mice. Nature **377:** 539–544

89 Sieg D. J., Hauck C. R. and Schlaepfer D. D. (1999) Required role of focal adhesion kinase (FAK) for integrin-stimulated cell migration. J. Cell Sci. **112 (Pt 16):** 2677–2691

90 Hannigan G. E., Leung-Hagesteijn C., Fitz-Gibbon L., Coppolino M. G., Radeva G., Filmus J. et al. (1996) Regulation of cell adhesion and anchorage-dependent growth by a new beta 1-integrin-linked protein kinase. Nature **379:** 91–96

91 Pasquet J. M., Noury M. and Nurden A. T. (2002) Evidence that the platelet integrin alphaIIb beta3 is regulated by the integrin-linked kinase, ILK, in a PI3-kinase dependent pathway. Thromb. Haemost. **88:** 115–122

92 Tu Y., Huang Y., Zhang Y., Hua Y. and Wu C. (2001) A new focal adhesion protein that interacts with integrin-linked kinase and regulates cell adhesion and spreading. J. Cell Biol. **153:** 585–598

93 Yamaji S., Suzuki A., Sugiyama Y., Koide Y., Yoshida M., Kanamori H. et al. (2001) A novel integrin-linked kinase-binding protein, affixin, is involved in the early stage of cell-substrate interaction. J. Cell Biol. **153:** 1251–1264

94 Nikolopoulos S. N. and Turner C. E. (2001) Integrin-linked kinase (ILK) binding to paxillin LD1 motif regulates ILK localization to focal adhesions. J. Biol. Chem. **276:** 23499–23505

95 Tu,Y., Li F., Goicoechea S. and Wu C. (1999) The LIM-only protein PINCH directly interacts with integrin-linked kinase and is recruited to integrin-rich sites in spreading cells. Mol. Cell Biol. **19:** 2425–2434

96 Zhang Y., Chen K., Guo L. and Wu C. (2002) Characterization of PINCH-2, a new focal adhesion protein that regulates the PINCH-1-ILK interaction, cell spreading and migration. J. Biol. Chem. **277:** 38328–38338

97 Braun A., Bordoy R., Stanchi F., Moser M., Kostka G. G., Ehler E. et al. (2003) PINCH2 is a new five LIM domain protein, homologous to PINCHand localized to focal adhesions. Exp. Cell Res. **284:** 239–250

98 Velyvis A., Yang Y., Wu C. and Qin J. (2001) Solution structure of the focal adhesion adaptor PINCH LIM1 domain and characterization of its interaction with the integrin-linked kinase ankyrin repeat domain. J. Biol. Chem. **276:** 4932–4939

99 Velyvis A., Vaynberg J., Yang Y., Vinogradova O., Zhang Y., Wu C. et al. (2003) Structural and functional insights into PINCH LIM4 domain-mediated integrin signaling. Nat. Struct. Biol. **10:** 558–564

100 Leung-Hagesteijn C., Mahendra A., Naruszewicz I. and Hannigan G. E. (2001) Modulation of integrin signal transduction by ILKAP, a protein phosphatase 2C associating with the integrin-linked kinase, ILK1. EMBO J. **20:** 2160–2170

101 Delcommenne M., Tan C., Gray V., Rue L., Woodgett J. and Dedhar S. (1998) Phosphoinositide-3-OH kinase-dependent regulation of glycogen synthase kinase 3 and protein kinase B/AKT by the integrin-linked kinase. Proc. Natl. Acad. Sci. USA **95:** 11211–11216

102 Persad S., Attwell S., Gray V., Mawji N., Deng J. T., Leung D. et al. (2001) Regulation of protein kinase B/Akt-serine 473 phosphorylation by integrin-linked kinase: critical roles for kinase activity and amino acids arginine 211 and serine 343. J. Biol. Chem. **276:** 27462–27469

103 Grashoff C., Thievessen I., Lorenz K., Ussar S. and Fassler R. (2004) Integrin-linked kinase: integrin's mysterious partner. Curr. Opin. Cell Biol. **16:** 565–571

104 Fukuda T., Chen K., Shi X. and Wu C. (2003) PINCH-1 is an obligate partner of integrin-linked kinase (ILK) functioning in cell shape modulation, motility and survival. J. Biol. Chem. **278:** 51324–51333

105 Yamaji S., Suzuki A., Kanamori H., Mishima W., Yoshimi R., Takasaki H. et al. (2004) Affixin interacts with alpha-actinin and mediates integrin signaling for reorganization of F-actin induced by initial cell-substrate interaction. J. Cell Biol. **165:** 539–551

106 Sakai T., Li S., Docheva D., Grashoff C., Sakai K., Kostka G. et al. (2003) Integrin-linked kinase (ILK) is required for polarizing the epiblast, cell adhesion, and controlling actin accumulation. Genes Dev. **17:** 926–940

107 Grashoff C., Aszodi A., Sakai T., Hunziker E. B. and Fassler R. (2003) Integrin-linked kinase regulates chondrocyte shape and proliferation. EMBO Rep. **4:** 432–438

108 Terpstra L., Prud'homme J., Arabian A., Takeda S., Karsenty G., Dedhar S. et al. (2003) Reduced chondrocyte proliferation and chondrodysplasia in mice lacking the integrin-linked kinase in chondrocytes. J. Cell Biol. **162:** 139–148

109 Wheeler G. N. and Hynes R. O. (2001) The cloning, genomic organization and expression of the focal contact protein paxillin in *Drosophila*. Gene **262:** 291–299

110 Gonczy P., Echeverri C., Oegema K., Coulson A., Jones S. J., Copley R. R. et al. (2000) Functional genomic analysis of cell division in *C. elegans* using RNAi of genes on chromosome III. Nature **408:** 331–336

111 Mazaki Y., Hashimoto S. and Sabe H. (1997) Monocyte cells and cancer cells express novel paxillin isoforms with different binding properties to focal adhesion proteins. J. Biol. Chem. **272:** 7437–7444

112 Chen L. M., Bailey D. and Fernandez-Valle C. (2000) Association of beta 1 integrin with focal adhesion kinase and paxillin in differentiating Schwann cells. J. Neurosci. **20:** 3776–3784

113 Liu S., Thomas S. M., Woodside D. G., Rose D. M., Kiosses W. B., Pfaff M. et al. (1999) Binding of paxillin to alpha4 integrins modifies integrin-dependent biological responses. Nature **402:** 676–681

114 Brown M. C., Perrotta J. A. and Turner C. E. (1996) Identification of LIM3 as the principal determinant of paxillin focal adhesion localization and characterization of a novel motif on paxillin directing vinculin and focal adhesion kinase binding. J. Cell Biol. **135:** 1109–1123

115 Nikolopoulos S. N. and Turner C. E. (2000) Actopaxin, a new focal adhesion protein that binds paxillin LD motifs and actin and regulates cell adhesion. J. Cell Biol. **151:** 1435–1448

116 Hashimoto S., Tsubouchi A., Mazaki Y. and Sabe H. (2001) Interaction of paxillin with p21-activated Kinase (PAK). Association of paxillin alpha with the kinase-inactive and the Cdc42-activated forms of PAK3. J. Biol. Chem. **276:** 6037–6045

117 Zhao Z. S., Manser E., Loo T. H. and Lim L. (2000) Coupling of PAK-interacting exchange factor PIX to GIT1 promotes focal complex disassembly. Mol. Cell Biol. **20:** 6354–6363

118 West K. A., Zhang H., Brown M. C., Nikolopoulos S. N., Riedy M. C., Horwitz A. F. et al. (2001) The LD4 motif of paxillin regulates cell spreading and motility through an interaction with paxillin kinase linker (PKL). J. Cell Biol. **154:** 161–176

119 Foletta V. C., Moussi N., Sarmiere P. D., Bamburg J. R. and Bernard O. (2004) LIM kinase 1, a key regulator of actin dynamics, is widely expressed in embryonic and adult tissues. Exp. Cell Res. **294:** 392–405

120 Schaller M. D. and Schaefer E. M. (2001) Multiple stimuli induce tyrosine phosphorylation of the Crk-binding sites of paxillin. Biochem. J. **360:** 57–66

121 Richardson A. and Parsons T. (1996) A mechanism for regulation of the adhesion-associated protein tyrosine kinase pp125FAK. Nature **380:** 538–540

122 Klinghoffer R. A., Sachsenmaier C., Cooper J. A. and Soriano P. (1999) Src family kinases are required for integrin but not PDGFR signal transduction. EMBO J. **18:** 2459–2471

123 Hagel M., George E. L., Kim A., Tamimi R., Opitz S. L., Turner C. E. et al. (2002) The adaptor protein paxillin is essential for normal development in the mouse and is a critical

transducer of fibronectin signaling. Mol. Cell Biol. **22:** 901–915
124 Laukaitis C. M., Webb D. J., Donais K. and Horwitz A. F. (2001) Differential dynamics of alpha 5 integrin, paxillin and alpha-actinin during formation and disassembly of adhesions in migrating cells. J. Cell Biol. **153:** 1427–1440
125 Webb D. J., Donais K., Whitmore L. A., Thomas S. M., Turner C. E., Parsons J. T. et al. (2004) FAK-Src signalling through paxillin, ERK and MLCK regulates adhesion disassembly. Nat. Cell Biol. **6:** 154–161
126 Carragher N. O., Westhoff M. A., Fincham V. J., Schaller M. D. and Frame M. C. (2003) A novel role for FAK as a protease-targeting adaptor protein: regulation by p42 ERK and Src. Curr. Biol. **13:** 1442–1450
127 Westhoff M. A., Serrels B., Fincham V. J., Frame M. C. and Carragher N. O. (2004) SRC-mediated phosphorylation of focal adhesion kinase couples actin and adhesion dynamics to survival signaling. Mol. Cell Biol. **24:** 8113–813.
128 Franco S., Perrin B. and Huttenlocher A. (2004) Isoform specific function of calpain 2 in regulating membrane protrusion. Exp. Cell Res. **299:** 179–187
129 Otey C. A., Pavalko F. M. and Burridge K. (1990) An interaction between alpha-actinin and the beta 1 integrin subunit in vitro. J. Cell Biol. **111:** 721–729
130 Sampath R., Gallagher P. J. and Pavalko F. M. (1998) Cytoskeletal interactions with the leukocyte integrin beta2 cytoplasmic tail. Activation-dependent regulation of associations with talin and alpha-actinin. J. Biol. Chem. **273:** 33588–33594
131 Goldmann W. H. (2000) Kinetic determination of focal adhesion protein formation. Biochem. Biophys. Res. Commun. **271:** 553–557
132 Izaguirre G., Aguirre L., Hu Y. P., Lee H. Y., Schlaepfer D. D., Aneskievich B. J. et al. (2001) The cytoskeletal/non-muscle isoform of alpha-actinin is phosphorylated on its actin-binding domain by the focal adhesion kinase. J. Biol. Chem. **276:** 28676–28685
133 Otey C. A. and Carpen O. (2004) Alpha-actinin revisited: a fresh look at an old player. Cell Motil. Cytoskeleton **58:** 104–111
134 Wachsstock D. H., Wilkins J. A. and Lin S. (1987) Specific interaction of vinculin with alpha-actinin. Biochem. Biophys. Res. Commun. **146:** 554–560
135 Crawford A. W. and Beckerle M. C. (1991) Purification and characterization of zyxin, an 82,000-dalton component of adherens junctions. J. Biol. Chem. **266:** 5847–5853
136 Shibasaki F., Fukami K., Fukui Y. and Takenawa T. (1994) Phosphatidylinositol 3-kinase binds to alpha-actinin through the p85 subunit. Biochem. J. **302 (Pt 2):** 551–557
137 Gluck U. and Ben Ze'ev A. (1994) Modulation of alpha-actinin levels affects cell motility and confers tumorigenicity on 3T3 cells. J. Cell Sci. **107 (Pt 7):** 1773–1782
138 Rajfur Z., Roy P., Otey C., Rome, L. and Jacobson K. (2002) Dissecting the link between stress fibres and focal adhesions by CALI with EGFP fusion proteins. Nat. Cell Biol. **4:** 286–293
139 van der Flier A. and Sonnenberg A. (2001) Structural and functional aspects of filamins. Biochim. Biophys. Acta **1538:** 99–117
140 Himmel M., Van Der Ven P. F., Stocklein W. and Furst D. O. (2003) The limits of promiscuity: isoform-specific dimerization of filamins. Biochemistry **42:** 430–439
141 Ohta Y., Suzuki N., Nakamura S., Hartwig J. H. and Stossel T. P. (1999) The small GTPase RalA targets filamin to induce filopodia. Proc. Natl. Acad. Sci. USA **96:** 2122–2128
142 Bellanger J. M., Astier C., Sardet C., Ohta Y., Stossel T. P. and Debant A. (2000) The Rac1- and RhoG-specific GEF domain of Trio targets filamin to remodel cytoskeletal actin. Nat. Cell Biol. **2:** 888–892
143 Ueda K., Ohta Y. and Hosoya H. (2003) The carboxy-terminal pleckstrin homology domain of ROCK interacts with filamin-A. Biochem. Biophys. Res. Commun. **301:** 886–890
144 Tu Y., Wu S., Shi X., Chen K. and Wu C. (2003) Migfilin and Mig-2 link focal adhesions to filamin and the actin cytoskeleton and function in cell shape modulation. Cell **113:** 37–47
145 D'Addario M., Arora P. D., Ellen R. P. and McCulloch C. A. (2002) Interaction of p38 and Sp1 in a mechanical force-induced, beta 1 integrin-mediated transcriptional circuit that regulates the actin-binding protein filamin-A. J. Biol. Chem. **277:** 47541–4755.
146 Calderwood D. A., Huttenlocher A., Kiosses W. B., Rose D. M., Woodside D. G., Schwartz M. A. et al. (2001) Increased filamin binding to beta-integrin cytoplasmic domains inhibits cell migration. Nat. Cell Biol. **3:** 1060–1068
147 Fox J. W., Lamperti E. D., Eksioglu Y. Z., Hong S. E., Feng Y., Graham D. A. et al. (1998) Mutations in filamin 1 prevent migration of cerebral cortical neurons in human periventricular heterotopia. Neuron **21:** 1315–1325
148 Lo S. H. (2004) Tensin. Int. J. Biochem. Cell Biol. **36:** 31–34
149 Calderwood D. A., Fujioka Y., de Pereda J. M., Garcia-Alvarez B., Nakamoto T., Margolis B. et al. (2003) Integrin beta cytoplasmic domain interactions with phosphotyrosine-binding domains: a structural prototype for diversity in integrin signaling. Proc. Natl. Acad. Sci. USA **100:** 2272–2277
150 Davis S., Lu M. L., Lo S. H., Lin S., Butler J. A., Druker B. J. et al. (1991) Presence of an SH2 domain in the actin-binding protein tensin. Science **252:** 712–715
151 Auger K. R., Songyang Z., Lo S. H., Roberts T. M. and Chen L. B. (1996) Platelet-derived growth factor-induced formation of tensin and phosphoinositide 3-kinase complexes. J. Biol. Chem. **271:** 23452–23457
152 Lo S. H., Yu Q. C., Degenstein L., Chen L. B. and Fuchs E. (1997) Progressive kidney degeneration in mice lacking tensin. J. Cell Biol. **136:** 1349–1361
153 Ishii A. and Lo S. H. (2001) A role of tensin in skeletal-muscle regeneration. Biochem. J. **356:** 737–745
154 Chen H., Duncan I. C., Bozorgchami H. and Lo S. H. (2002) Tensin1 and a previously undocumented family member, tensin2, positively regulate cell migration. Proc. Natl. Acad. Sci. USA **99:** 733–738
155 Arias-Salgado E. G., Lizano S., Sarkar S., Brugge J. S., Ginsberg M. H. and Shattil S. J. (2003) Src kinase activation by direct interaction with the integrin beta cytoplasmic domain. Proc. Natl. Acad. Sci. USA **100:** 13298–13302
156 McCahill A., Warwicker J., Bolger G. B., Houslay M. D. and Yarwood S. J. (2002) The RACK1 scaffold protein: a dynamic cog in cell response mechanisms. Mol. Pharmacol. **62:** 1261–1273
157 Besson A., Wilson T. L. and Yong V. W. (2002) The anchoring protein RACK1 links protein kinase Cepsilon to integrin beta chains. Requirements for adhesion and motility. J. Biol. Chem. **277:** 22073–22084
158 Chang B. Y., Conroy K. B., Machleder E. M. and Cartwright C. A. (1998) RACK1, a receptor for activated C kinase and a homolog of the beta subunit of G proteins, inhibits activity of src tyrosine kinases and growth of NIH 3T3 cells. Mol. Cell Biol. **18:** 3245–3256
159 Cox E. A., Bennin D., Doan A. T., O'Toole T. and Huttenlocher A. (2003) RACK1 regulates integrin-mediated adhesion, protrusion, and chemotactic cell migration via its Src-binding site. Mol. Biol. Cell **14:** 658–669
160 Zhang H., Berg J. S., Li Z., Wang Y., Lang P., Sousa A. D. et al. (2004) Myosin-X provides a motor-based link between integrins and the cytoskeleton. Nat. Cell Biol. **6:** 523–531
161 Cornillon S., Pech E., Benghezal M., Ravanel K., Gaynor E., Letourneur F. et al. (2000) Phg1p is a nine-transmembrane protein superfamily member involved in dictyostelium adhesion and phagocytosis. J. Biol. Chem. **275:** 34287–34292

162 Fey P., Stephens S., Titus M. A. and Chisholm R. L. (2002) SadA, a novel adhesion receptor in *Dictyostelium*. J. Cell Biol. **159:** 1109–1119

163 Abraham V. C., Krishnamurthi V., Taylor D. L. and Lanni F. (1999) The actin-based nanomachine at the leading edge of migrating cells. Biophys. J. **77:** 1721–1732

164 Upadhyaya A., Chabot J. R., Andreeva A., Samadani A. and van Oudenaarden A. (2003) Probing polymerization forces by using actin-propelled lipid vesicles. Proc. Natl. Acad. Sci. USA **100:** 4521–4526

165 Marcy Y., Prost J., Carlier M. F. and Sykes C. (2004) Forces generated during actin-based propulsion: a direct measurement by micromanipulation. Proc. Natl. Acad. Sci. USA **101:** 5992–5997

166 Machesky L. M., Atkinson S. J., Ampe C., Vandekerckhove J. and Pollard T. D. (1994) Purification of a cortical complex containing two unconventional actins from *Acanthamoeba* by affinity chromatography on profilin-agarose. J. Cell Biol. **127:** 107–115

167 Mullins R. D., Heuser J. A. and Pollard T. D. (1998) The interaction of Arp2/3 complex with actin: nucleation, high affinity pointed end capping and formation of branching networks of filaments. Proc. Natl. Acad. Sci. USA **95:** 6181–6186

168 Pantaloni D., Boujemaa R., Didry D., Gounon P. and Carlier M. F. (2000) The Arp2/3 complex branches filament barbed ends: functional antagonism with capping proteins. Nat. Cell Biol. **2:** 385–391

169 Loisel T. P., Boujemaa R., Pantaloni D. and Carlier M. F. (1999) Reconstitution of actin-based motility of *Listeria* and *Shigella* using pure proteins. Nature **401:** 613–616

170 Wiesner S., Helfer E., Didry D., Ducouret G., Lafuma F., Carlier M. F. et al. (2003) A biomimetic motility assay provides insight into the mechanism of actin-based motility. J. Cell Biol. **160:** 387–398

171 Didry D., Carlier M. F. and Pantaloni D. (1998) Synergy between actin depolymerizing factor/cofilin and profilin in increasing actin filament turnover. J. Biol. Chem. **273:** 25602–25611

172 Miki H. and Takenawa T. (2003) Regulation of actin dynamics by WASP family proteins. J. Biochem. (Tokyo) **134:** 309–313

173 Stradal T. E., Rottner K., Disanza A., Confalonieri S., Innocenti M. and Scita G. (2004) Regulation of actin dynamics by WASP and WAVE family proteins. Trends Cell Biol. **14:** 303–311

174 Rohatgi R., Ma L., Miki H., Lopez M., Kirchhausen T., Takenawa T. et al. (1999) The interaction between N-WASP and the Arp2/3 complex links Cdc42-dependent signals to actin assembly. Cell **97:** 221–231

175 Kim A. S., Kakalis L. T., Abdul-Manan N., Liu G. A. and Rosen M. K. (2000) Autoinhibition and activation mechanisms of the Wiskott-Aldrich syndrome protein. Nature **404:** 151–158

176 Kunda P., Craig G., Dominguez V. and Baum B. (2003) Abi, Sra1, and Kette control the stability and localization of SCAR/WAVE to regulate the formation of actin-based protrusions. Curr. Biol. **13:** 1867–1875

177 Innocenti M., Zucconi A., Disanza A., Frittoli E., Areces L. B., Steffen A. et al. (2004) Abi1 is essential for the formation and activation of a WAVE2 signalling complex. Nat. Cell Biol. **6:** 319–327

178 Steffen A., Rottner K., Ehinger J., Innocenti M., Scita G., Wehland J. et al. (2004) Sra-1 and Nap1 link Rac to actin assembly driving lamellipodia formation. EMBO J. **23:** 749–759

179 Miki H., Suetsugu S. and Takenawa T. (1998) WAVE, a novel WASP-family protein involved in actin reorganization induced by Rac. EMBO J. **17:** 6932–6941

180 Yan C., Martinez-Quiles N., Eden S., Shibata T., Takeshima F., Shinkura R. et al. (2003) WAVE2 deficiency reveals distinct roles in embryogenesis and Rac-mediated actin-based motility. EMBO J. **22:** 3602–3612

181 Snapper S. B., Takeshima F., Anton I., Liu C. H., Thomas S. M., Nguyen D. et al. (2001) N-WASP deficiency reveals distinct pathways for cell surface projections and microbial actin-based motility. Nat. Cell Biol. **3:** 897–904

182 Watanabe N., Kato T., Fujita A., Ishizaki T. and Narumiya S. (1999) Cooperation between mDia1 and ROCK in Rho-induced actin reorganization. Nat. Cell Biol. **1:** 136–143

183 Cory G. O., Garg R., Cramer R. and Ridley A. J. (2002) Phosphorylation of tyrosine 291 enhances the ability of WASp to stimulate actin polymerization and filopodium formation. Wiskott-Aldrich Syndrome protein. J. Biol. Chem. **277:** 45115–4512.

184 Suetsugu S., Hattori M., Miki H., Tezuka T., Yamamoto T., Mikoshiba K. et al. (2002) Sustained activation of N-WASP through phosphorylation is essential for neurite extension. Dev. Cell **3:** 645–658

185 Mizutani K., Miki H., He H., Maruta H. and Takenawa T. (2002) Essential role of neural Wiskott-Aldrich syndrome protein in podosome formation and degradation of extracellular matrix in src-transformed fibroblasts. Cancer Res. **62:** 669–674

186 Linder S., Nelson D., Weiss M. and Aepfelbacher M. (1999) Wiskott-Aldrich syndrome protein regulates podosomes in primary human macrophages. Proc. Natl. Acad. Sci. USA **96:** 9648–9653

187 Wu X., Suetsugu S., Cooper L. A., Takenawa T. and Guan J. L. (2004) Focal adhesion kinase regulation of N-WASP subcellular localization and function. J. Biol. Chem. **279:** 9565–9576

188 Tu Y., Li F. and Wu C. (1998) Nck-2, a novel Src homology2/3-containing adaptor protein that interacts with the LIM-only protein PINCH and components of growth factor receptor kinase-signaling pathways. Mol. Biol. Cell **9:** 3367–3382

189 Uruno T., Liu J., Zhang P., Fan Y., Egile C., Li R. et al. (2001) Activation of Arp2/3 complex-mediated actin polymerization by cortactin. Nat. Cell Biol. **3:** 259–266

190 DeMali K. A., Barlow, C. A. and Burridge K. (2002) Recruitment of the Arp2/3 complex to vinculin: coupling membrane protrusion to matrix adhesion. J. Cell Biol. **159:** 881–891

191 Vadlamudi R. K., Li F., Barnes C. J., Bagheri-Yarmand R. and Kumar R. (2004) p41-Arc subunit of human Arp2/3 complex is a p21-activated kinase-1-interacting substrate. EMBO Rep. **5:** 154–160

192 Raftopoulou M. and Hall A. (2004) Cell migration: Rho GTPases lead the way. Dev. Biol. **265:** 23–32

193 Price L. S., Leng J., Schwartz M. A. and Bokoch G. M. (1998) Activation of Rac and Cdc42 by integrins mediates cell spreading. Mol. Biol. Cell **9:** 1863–1871

194 Ren X. D., Kiosses W. B. and Schwartz M. A. (1999) Regulation of the small GTP-binding protein Rho by cell adhesion and the cytoskeleton. EMBO J. **18:** 578–585

195 Hirsch E., Barberis L., Brancaccio M., Azzolino O., Xu D., Kyriakis J. M. et al. (2002) Defective Rac-mediated proliferation and survival after targeted mutation of the beta1 integrin cytodomain. J. Cell Biol. **157:** 481–492

196 Berrier A. L., Martinez R., Bokoch G. M. and LaFlamme S. E. (2002) The integrin beta tail is required and sufficient to regulate adhesion signaling to Rac1. J. Cell Sci. **115:** 4285–4291

197 Scita G., Nordstrom J., Carbone R., Tenca P., Giardina G., Gutkind S. et al. (1999) EPS8 and E3B1 transduce signals from Ras to Rac. Nature **401:** 290–293

198 Hsia D. A., Mitra S. K., Hauck C. R., Streblow D. N., Nelson J. A., Ilic D. et al. (2003) Differential regulation of cell motility and invasion by FAK. J. Cell Biol. **160:** 753–767

199 Turner C. E., Brown M. C., Perrotta J. A., Riedy M. C., Nikolopoulos S. N., McDonald A. R. et al. (1999) Paxillin LD4 motif binds PAK and PIX through a novel 95-kD ankyrin repeat, ARF-GAP protein: a role in cytoskeletal remodeling. J. Cell Biol. **145:** 851–863

200 Rosenberger G., Jantke I., Gal A. and Kutsche K. (2003) Interaction of alphaPIX (ARHGEF6) with beta-parvin (PARVB) suggests an involvement of alphaPIX in integrin-mediated signaling. Hum. Mol. Genet. **12:** 155–167
201 del Pozo M. A., Kiosses W. B., Alderson N. B., Meller N., Hahn K. M. and Schwartz M. A. (2002) Integrins regulate GTP-Rac localized effector interactions through dissociation of Rho-GDI. Nat. Cell Biol. **4:** 232–239
202 del Pozo M. A., Alderson N. B., Kiosses W. B., Chiang H. H., Anderson R. G. and Schwartz M. A. (2004) Integrins regulate Rac targeting by internalization of membrane domains. Science **303:** 839–842
203 Arthur W. T., Petch L. A. and Burridge K. (2000) Integrin engagement suppresses RhoA activity via a c-Src-dependent mechanism. Curr. Biol. **10:** 719–722
204 Tsubouchi A., Sakakura J., Yagi R., Mazaki Y., Schaefer E., Yano H. et al. (2002) Localized suppression of RhoA activity by Tyr31/118-phosphorylated paxillin in cell adhesion and migration. J. Cell Biol. **159:** 673–683
205 Palazzo A. F., Eng C. H., Schlaepfer D. D., Marcantonio E. E. and Gundersen G. G. (2004) Localized stabilization of microtubules by integrin- and FAK-facilitated Rho signaling. Science **303:** 836–839
206 Reif K., Nobes C. D., Thomas G., Hall A. and Cantrell D. A. (1996) Phosphatidylinositol 3-kinase signals activate a selective subset of Rac/Rho-dependent effector pathways. Curr. Biol. **6:** 1445–1455
207 Benard V., Bohl B. P. and Bokoch G. M. (1999) Characterization of rac and cdc42 activation in chemoattractant-stimulated human neutrophils using a novel assay for active GTPases. J. Biol. Chem. **274:** 13198–13204
208 Hawkins P. T., Eguinoa A., Qiu R. G., Stokoe D., Cooke F. T., Walters R. et al. (1995) PDGF stimulates an increase in GTP-Rac via activation of phosphoinositide 3-kinase. Curr. Biol. **5:** 393–403
209 Nakagawa M., Fukata M., Yamaga M., Itoh N. and Kaibuchi K. (2001) Recruitment and activation of Rac1 by the formation of E-cadherin-mediated cell-cell adhesion sites. J. Cell Sci. **114:** 1829–1838
210 Bokoch G. M., Vlahos C. J., Wang Y., Knaus U. G. and Traynor-Kaplan A. E. (1996) Rac GTPase interacts specifically with phosphatidylinositol 3-kinase. Biochem. J. **315 (Pt 3):** 775–779
211 Zheng Y., Bagrodia S. and Cerione R. A. (1994) Activation of phosphoinositide 3-kinase activity by Cdc42Hs binding to p85. J. Biol. Chem. **269:** 18727–18730
212 Schmidt A. and Hall A. (2002) Guanine nucleotide exchange factors for Rho GTPases: turning on the switch. Genes Dev. **16:** 1587–1609
213 Funamoto S., Meili R., Lee S., Parry L. and Firtel R. A. (2002) Spatial and temporal regulation of 3-phosphoinositides by PI 3-kinase and PTEN mediates chemotaxis. Cell **109:** 611–623
214 Merlot S. and Firtel R. A. (2003) Leading the way: directional sensing through phosphatidylinositol 3-kinase and other signaling pathways. J. Cell Sci. **116:** 3471–3478
215 Reiske H. R., Kao S. C., Cary L. A., Guan J. L., Lai J. F. and Chen H. C. (1999) Requirement of phosphatidylinositol 3-kinase in focal adhesion kinase-promoted cell migration. J. Biol. Chem. **274:** 12361–12366
216 Krause M., Leslie J. D., Stewart M., Lafuente E. M., Valderrama F., Jagannathan R. et al. (2004) Lamellipodin, an Ena/VASP ligand, is implicated in the regulation of lamellipodial dynamics. Dev. Cell **7:** 571–583
217 Auger K. R., Serunian L. A., Soltoff,S. P., Libby P. and Cantley L. C. (1989) PDGF-dependent tyrosine phosphorylation stimulates production of novel polyphosphoinositides in intact cells. Cell **57:** 167–175
218 Greenwood J. A., Theibert A. B., Prestwich G. D. and Murphy-Ullrich J. E. (2000) Restructuring of focal adhesion plaques by PI 3-kinase. Regulation by PtdIns (3,4,5)-p(3) binding to alpha-actinin. J. Cell Biol. **150:** 627–642
219 Furuhashi K., Inagaki M., Hatano S., Fukami K. and Takenawa T. (1992) Inositol phospholipid-induced suppression of F-actin-gelating activity of smooth muscle filamin. Biochem. Biophys. Res. Commun. **184:** 1261–1265
220 Fukami K., Endo T., Imamura M. and Takenawa, T. (1994) alpha-Actinin and vinculin are PIP2-binding proteins involved in signaling by tyrosine kinase. J. Biol. Chem. **269:** 1518–1522.
221 Weekes J., Barry S. T. and Critchley D. R. (1996) Acidic phospholipids inhibit the intramolecular association between the N- and C-terminal regions of vinculin, exposing actin-binding and protein kinase C phosphorylation sites. Biochem. J. **314(Pt 3):** 827–832
222 Steimle P. A., Hoffert J. D., Adey N. B. and Craig S. W. (1999) Polyphosphoinositides inhibit the interaction of vinculin with actin filaments. J. Biol. Chem. **274:** 18414–18420
223 Fukami K., Furuhashi K., Inagaki M., Endo T., Hatano S. and Takenawa T. (1992) Requirement of phosphatidylinositol 4,5-bisphosphate for alpha-actinin function. Nature **359:** 150–152
224 Fraley T. S., Tran T. C., Corgan A. M., Nash C. A., Hao J., Critchley D. R. et al. (2003) Phosphoinositide binding inhibits alpha-actinin bundling activity. J. Biol. Chem. **278:** 24039–24045
225 McNamee H. P., Ingber D. E. and Schwartz M. A. (1993) Adhesion to fibronectin stimulates inositol lipid synthesis and enhances PDGF-induced inositol lipid breakdown. J. Cell Biol. **121:** 673–678
226 Ling K., Doughman R. L., Iyer V. V., Firestone A. J., Bairstow S. F., Mosher D. F. et al. (2003) Tyrosine phosphorylation of type Igamma phosphatidylinositol phosphate kinase by Src regulates an integrin-talin switch. J. Cell Biol. **163:** 1339–1349.
227 Itoh T., Ishihara H., Shibasaki Y., Oka Y. and Takenawa T. (2000) Autophosphorylation of type I phosphatidylinositol phosphate kinase regulates its lipid kinase activity. J. Biol. Chem. **275:** 19389–19394
228 Park S. J., Itoh T. and Takenawa T. (2001) Phosphatidylinositol 4-phosphate 5-kinase type I is regulated through phosphorylation response by extracellular stimuli. J. Biol. Chem. **276:** 4781–4787
229 Barsukov I. L., Prescot A., Bate N., Patel B., Floyd D. N., Bhanji N. et al. (2003) Phosphatidylinositol phosphate kinase type 1gamma and beta1-integrin cytoplasmic domain bind to the same region in the talin FERM domain. J. Biol. Chem. **278:** 31202–31209
230 Yin H. L. and Janmey P. A. (2003) Phosphoinositide regulation of the actin cytoskeleton. Annu. Rev. Physiol **65:** 761–789
231 Tolias K. F., Cantley L. C. and Carpenter C. L. (1995) Rho family GTPases bind to phosphoinositide kinases. J. Biol. Chem. **270:** 17656–17659
232 Tolias K. F., Couvillon A. D., Cantley L. C. and Carpenter C. L. (1998) Characterization of a Rac1- and RhoGDI-associated lipid kinase signaling complex. Mol. Cell Biol. **18:** 762–770
233 Weernink P. A., Meletiadis K., Hommeltenberg S., Hinz M., Ishihara H., Schmidt M. et al. (2004) Activation of type I phosphatidylinositol 4-phosphate 5-kinase isoforms by the Rho GTPases, RhoA, Rac1, and Cdc42. J. Biol. Chem. **279:** 7840–7849
234 Weernink P. A., Guo Y., Zhang C., Schmidt M., Eichel-Streiber C. and Jakobs K. H. (2000) Control of cellular phosphatidylinositol 4,5-bisphosphate levels by adhesion signals and rho GTPases in NIH 3T3 fibroblasts involvement of both phosphatidylinositol-4-phosphate 5-kinase and phospholipase C. Eur. J. Biochem. **267:** 5237–5246
235 Yang S. A., Carpenter C. L. and Abrams C. S. (2004) Rho and Rho-kinase mediate thrombin-induced phosphatidylinositol 4-phosphate 5-kinase trafficking in platelets. J. Biol. Chem. **279:** 42331–42336

236 Chatah N. E. and Abrams C. S. (2001) G-protein-coupled receptor activation induces the membrane translocation and activation of phosphatidylinositol-4-phosphate 5-kinase I alpha by a Rac- and Rho-dependent pathway. J. Biol. Chem. **276:** 34059–34065

237 Bouvard D., Brakebusch C., Gustafsson E., Aszodi A., Bengtsson T., Berna A. et al. (2001) Functional consequences of integrin gene mutations in mice. Circ. Res. **89:** 211–223

238 Fassler R. and Meyer M. (1995) Consequences of lack of beta 1 integrin gene expression in mice. Genes Dev. **9:** 1896–1908

239 Stephens L. E., Sutherland A. E., Klimanskaya I. V., Andrieux A., Meneses J., Pedersen R. A. et al. (1995) Deletion of beta 1 integrins in mice results in inner cell mass failure and peri-implantation lethality. Genes Dev. **9:** 1883–1895

240 White D. J., Puranen S., Johnson M. S. and Heino J. (2004) The collagen receptor subfamily of the integrins. Int. J. Biochem. Cell Biol. **36:** 1405–1410

241 Hodivala-Dilke K. M., McHugh K. P., Tsakiris D. A., Rayburn H., Crowley D., Ullman-Cullere M. et al. (1999) Beta3-integrin-deficient mice are a model for Glanzmann thrombasthenia showing placental defects and reduced survival. J. Clin. Invest **103:** 229–238

242 Reynolds L. E., Wyder L., Lively J. C., Taverna D., Robinson S. D., Huang X. et al. (2002) Enhanced pathological angiogenesis in mice lacking beta3 integrin or beta3 and beta5 integrins. Nat. Med. **8:** 27–34

243 Brooks P. C., Clark R. A. and Cheresh D. A. (1994) Requirement of vascular integrin alpha v beta 3 for angiogenesis. Science **264:** 569–571

244 Brooks P. C., Montgomery A. M., Rosenfeld M., Reisfeld R. A., Hu T., Klier G. et al. (1994) Integrin alpha v beta 3 antagonists promote tumor regression by inducing apoptosis of angiogenic blood vessels. Cell **79:** 1157–1164

245 Friedlander M., Brooks P. C., Shaffer R. W., Kincaid C. M., Varner J. A. and Cheresh D. A. (1995) Definition of two angiogenic pathways by distinct alpha v integrins. Science **270:** 1500–1502

246 Hynes R. O. (2002) A reevaluation of integrins as regulators of angiogenesis. Nat. Med. **8:** 918–921

247 Mercurio A. M., Rabinovitz I. and Shaw L. M. (2001) The alpha 6 beta 4 integrin and epithelial cell migration. Curr. Opin. Cell Biol. **13:** 541–545

248 Danen E. H., Sonneveld P., Brakebusch C., Fassler R. and Sonnenberg A. (2002) The fibronectin-binding integrins alpha5beta1 and alphavbeta3 differentially modulate RhoA-GTP loading, organization of cell matrix adhesions and fibronectin fibrillogenesis. J. Cell Biol. **159:** 1071–1086

249 Miao H., Li S., Hu Y. L., Yuan S., Zhao Y., Chen B. P. et al. (2002) Differential regulation of Rho GTPases by beta1 and beta3 integrins: the role of an extracellular domain of integrin in intracellular signaling. J. Cell Sci. **115:** 2199–2206

250 Chan B. M., Kassner P. D., Schiro J. A., Byers H. R., Kupper T. S. and Hemler M. E. (1992) Distinct cellular functions mediated by different VLA integrin alpha subunit cytoplasmic domains. Cell **68:** 1051–1060

251 Han J., Liu S., Rose D. M., Schlaepfer D. D., McDonld H., and Ginsberg M. H. (2001) Phosphorylation of the integrin alpha 4 cytoplasmic domain regulates paxillin binding. J. Biol. Chem. **276:** 40903–40909

252 Goldfinger L. E., Han J., Kiosses W. B., Howe A. K. and Ginsberg M. H. (2003) Spatial restriction of alpha4 integrin phosphorylation regulates lamellipodial stability and alpha4beta1-dependent cell migration. J. Cell Biol. **162:** 731–741

253 Belkin A. M., Retta S. F., Pletjushkina O. Y., Balzac F., Silengo L., Fassler R. et al. (1997) Muscle beta1D integrin reinforces the cytoskeleton-matrix link: modulation of integrin adhesive function by alternative splicing. J. Cell Biol. **139:** 1583–1595

254 Belkin A. M. and Retta S. F. (1998) beta1D integrin inhibits cell cycle progression in normal myoblasts and fibroblasts. J. Biol. Chem. **273:** 15234–15240

255 Moro L., Perlino E., Marra E., Languino,L. R. and Greco M. (2004) Regulation of beta1C and beta1A integrin expression in prostate carcinoma cells. J. Biol. Chem. **279:** 1692–1702

256 Blystone S. D., Lindberg F. P., Williams M. P., McHugh K. P., and Brown E. J. (1996) Inducible tyrosine phosphorylation of the beta3 integrin requires the alphaV integrin cytoplasmic tail. J. Biol. Chem. **271:** 31458–31462

257 Blystone S. D., Slater S. E., Williams M. P., Crow M. T. and Brown E. J. (1999) A molecular mechanism of integrin crosstalk: alphavbeta3 suppression of calcium/calmodulin-dependent protein kinase II regulates alpha5beta1 function. J. Cell Biol. **145:** 889–897

258 Law D. A., DeGuzman F. R., Heiser P., Ministri-Madrid K., Killeen N. and Phillips D. R. (1999) Integrin cytoplasmic tyrosine motif is required for outside-in alphaIIbbeta3 signalling and platelet function. Nature **401:** 808–811

259 Law D. A., Nannizzi-Alaimo L. and Phillips D. R. 1996) Outside-in integrin signal transduction. Alpha IIb beta 3-(GP IIb IIIa) tyrosine phosphorylation induced by platelet aggregation. J. Biol. Chem. **271:** 10811–10815

260 Chandhoke S. K., Williams M., Schaefer E., Zorn L. and Blystone S. D. (2004) Beta 3 integrin phosphorylation is essential for Arp3 organization into leukocyte alpha V beta 3-vitronectin adhesion contacts. J. Cell Sci. **117:** 1431–1441

261 Valmu L., Fagerholm S., Suila H. and Gahmberg C. G. (1999) The cytoskeletal association of CD11/CD18 leukocyte integrins in phorbol ester-activated cells correlates with CD18 phosphorylation. Eur. J. Immunol. **29:** 2107–2118

262 Fagerholm S., Morrice N., Gahmberg C. G. and Cohen P. (2002) Phosphorylation of the cytoplasmic domain of the integrin CD18 chain by protein kinase C isoforms in leukocytes. J. Biol. Chem. **277:** 1728–1738

263 Du X., Saido T. C., Tsubuki S., Indig F. E., Williams M. J. and Ginsberg M. H. (1995) Calpain cleavage of the cytoplasmic domain of the integrin beta 3 subunit. J. Biol. Chem. **270:** 26146–26151

264 Pfaff M., Du X. and Ginsberg M. H. (1999) Calpain cleavage of integrin beta cytoplasmic domains. FEBS Lett. **460:** 17–22

265 Glading A., Lauffenburger D. A. and Wells A. (2002) Cutting to the chase: calpain proteases in cell motility. Trends Cell Biol. **12:** 46–54

266 Sasaki H., Nagura K., Ishino M., Tobioka H., Kotani K. and Sasaki T. (1995) Cloning and characterization of cell adhesion kinase beta, a novel protein-tyrosine kinase of the focal adhesion kinase subfamily. J. Biol. Chem. **270:** 21206–21219

267 Lipinski C. A., Tran N. L., Bay C., Kloss J., McDonough W. S., Beaudry C. et al. (2003) Differential role of proline-rich tyrosine kinase 2 and focal adhesion kinase in determining glioblastoma migration and proliferation. Mol. Cancer Res. **1:** 323–332

268 Lipsky B. P., Beals C. R. and Staunton D. E. (1998) Leupaxin is a novel LIM domain protein that forms a complex with PYK2. J. Biol. Chem. **273:** 11709–11713

269 Muller U., Wang D., Denda S., Meneses J. J., Pedersen R. A., and Reichardt L. F. (1997) Integrin alpha8beta1 is critically important for epithelial-mesenchymal interactions during kidney morphogenesis. Cell **88:** 603–613