Dynamic Regulation of the Structure and Functions of Integrin Adhesions

Haguy Wolfenson,[1,2] Irena Lavelin,[1] and Benjamin Geiger[1,*]
[1]Department of Molecular Cell Biology, Weizmann Institute of Science, Rehovot 76100, Israel
[2]Current address: Department of Biological Sciences, Columbia University, New York, NY 10027, USA
*Correspondence: benny.geiger@weizmann.ac.il
http://dx.doi.org/10.1016/j.devcel.2013.02.012

Integrin-mediated cell adhesions to the extracellular matrix (ECM) contribute to tissue morphogenesis and coherence and provide cells with vital environmental cues. These apparently static structures display remarkable plasticity and dynamic properties: they exist in multiple, interconvertible forms that are constantly remodeled in response to changes in ECM properties, cytoskeletal organization, cell migration, and signaling processes. Thus, integrin-mediated environmental sensing enables cells to adapt to chemical and physical properties of the surrounding matrix by modulating their proliferation, differentiation, and survival. This intriguing interplay between the apparently robust structure of matrix adhesions and their highly dynamic properties is the focus of this article.

The History of Integrin Adhesion Research: From Early Structural Studies to Contemporary Functional Proteomics

Since the very early days of cell culture research more than 100 years ago, researchers have recognized the importance of cell adhesion to the extracellular environment and its essential role in cell survival, growth, and migration. As early as 1911, Ross G. Harrison noted that cells "require some form of solid support in order to carry out the growth process" (Harrison, 1911). A decade later, Warren H. Lewis wrote, "Cells that migrate out on the under surface of the cover-glass… are sticky for glass… not only the [cell] bodies but the cell processes as well possess this adhesive quality"; and, he added, "we may in time be able to measure the force of the adhesions in some way" (Lewis, 1922).

The observation that adhesions are located at the edges of lamellae was first made by Hubert B. Goodrich (Goodrich, 1924) and later corroborated by others (Chambers and Fell, 1931; Algard, 1953; Rappaport and Rappaport, 1968). However, as Albert Harris wrote in 1973, "perhaps because of the oddness of this observation, or perhaps because it was not the principal conclusion of any of the papers cited, the phenomenon has not become generally recognized, and its consequences and likely significance have never been fully explored" (Harris, 1973). Meanwhile, renewed interest during the 1950s in the discoveries of Francis Peyton Rous on the viral cause of cancer (Rous, 1911) led researchers to produce malignancies in cell culture (Temin and Rubin, 1958; Sanford et al., 1961) and highlighted the importance of cell adhesion in the so-called "contact inhibition" (Abercrombie and Heaysman, 1954) and "anchorage dependence" (Stoker et al., 1968) of nonmalignant cells.

It was only in the mid-1960s and early 1970s that researchers were able to view the focal nature of matrix adhesions and their precise locations, using interference reflection microscopy (IRM) (Curtis, 1964; Abercrombie and Dunn, 1975) and transmission electron microscopy (TEM) (Abercrombie et al., 1971; Revel and Wolken, 1973). These studies led to several important observations, including the distinction between focal adhesions (or focal contacts, as they were often called), which are located under the lamella, and close contacts, which are somewhat less tight and are broadly associated with the lamellipodium (Izzard and Lochner, 1976). These experiments also provided the earliest evidence that focal adhesions are connected to the cell's cytoskeleton (Izzard and Lochner, 1976; Heath and Dunn, 1978; Kreis et al., 1979) via actin stress fibers that take an active role in regulating adhesion (Rees et al., 1977).

Around the same time, fibronectin emerged as the major extracellular protein participating in the formation of focal adhesions (Hynes and Destree, 1978; Thom et al., 1979). Additional evidence further demonstrated that the two sets of fibrils—actin inside the cell and fibronectin on the outside—are physically connected (Heggeness et al., 1978; Hynes and Destree, 1978; Singer, 1979). These findings led to the conclusion that a transmembrane linker protein (a "fibronectin receptor") must exist, but it was not until 1987 that integrins were ultimately identified as the elusive receptors (Hynes, 1987). It was also recognized then that integrins operate as heterodimers composed of α and β subunits.

The molecular era of integrin adhesions began in the late 1970s and early 1980s, when vinculin and tyrosine-phosphorylated proteins were first shown to reside in these extracellular matrix (ECM) adhesions (Geiger, 1979; Burridge and Feramisco, 1980; Rohrschneider, 1980). These were followed by further discoveries of adhesion-related proteins, including structural proteins (such as paxillin, zyxin, α-actinin, and tensin), as well as signaling molecules (kinases such as FAK, Abl, and PKC, phosphatases such as SHP-2 and LAR-PTP, and other enzymes such as PI3-kinase and calpain II). The functional and molecular diversity of integrin adhesions was initially clarified in 2000 (Zamir et al., 2000), with distinctions drawn among focal adhesions, focal complexes, fibrillar adhesions, and podosomes (see examples in Figure 1). This body of knowledge was further expanded in 2007, when the ensemble of focal adhesion-associated proteins was defined as the "integrin adhesome" (Zaidel-Bar et al., 2007a) (see following section).
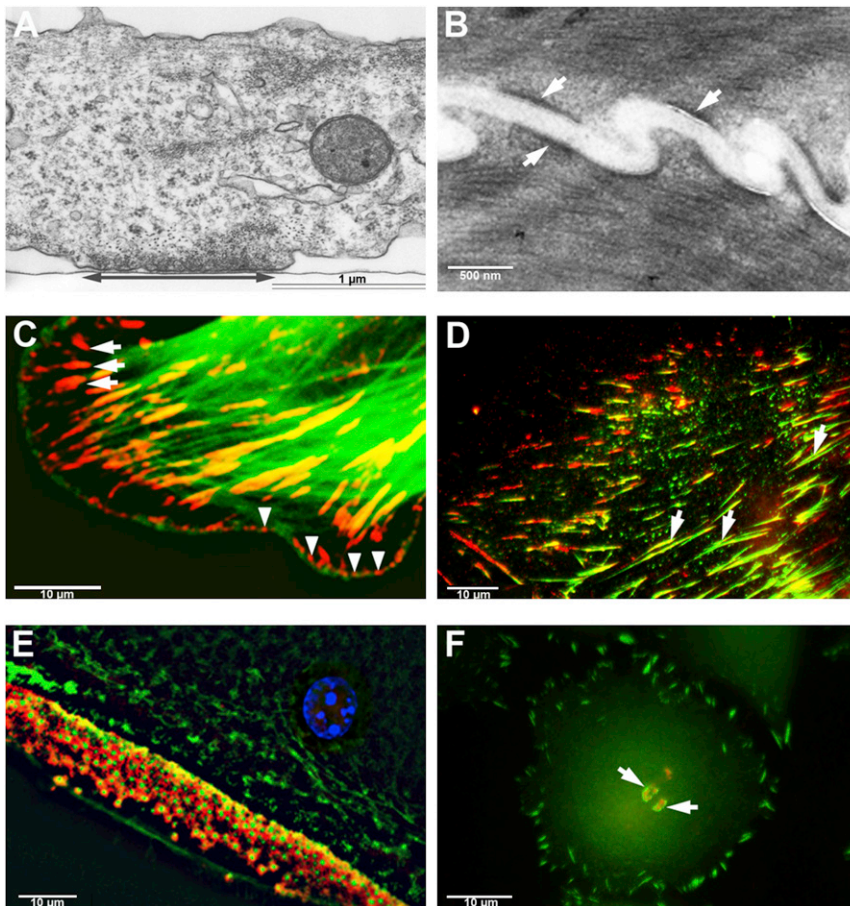
Figure 1. Diversity of Integrin-Mediated Cell Matrix Adhesion Structures as Viewed by Different Microscopy Techniques
(A) A transmission electron microscope (TEM) image of chicken lens cells in culture. The black arrow indicates a focal adhesion. (B) A cryo-EM image of chicken gizzard smooth muscle. Arrows indicate focal adhesions sites. (C) Focal adhesions (arrows) and focal complexes (arrowheads) formed by a human foreskin fibroblast stained for paxillin (red) and actin (green). (D) Fibrillar adhesions (arrows) formed by a WI38 human lung fibroblast, stained for tensin (red) and fibronectin (green). (E) Podosomes forming a "sealing zone" in a cultured osteoclast derived from murine bone marrow, stained for paxillin (red) and actin (green). (F) Invadopodia (arrows) formed by an A375 metastatic melanoma cell stained for vinculin (green) and TKS5 (red). Images in (A) and (B) were provided by Ilana Sabanay; images in (C) and (D) were provided by Tova Volberg; image in (E) was provided by Chen Luxenburg; image in (F) was provided by Or-Yam Shoshana.

The Integrin Adhesome: Molecular Diversity versus Functional Unity

During the past several decades, attempts to characterize the molecular components of integrin adhesions have yielded a long list of molecules that are known to be directly associated with the formation and regulation of focal adhesions and related structures. Efforts to place these molecules within a unifying functional framework resulted in some literature-based publications describing the complexity, order, and switchability of the integrin adhesome (Zaidel-Bar et al., 2007a; Zaidel-Bar and Geiger, 2010). These were complemented by detailed proteomic studies directly mapping the components of integrin adhesions and their interconnections (Humphries et al., 2009; Kuo et al., 2011; Schiller et al., 2011; for further reading, see Geiger and Zaidel-Bar, 2012).

Generally, the molecular components of the integrin adhesome (about 180 to date) can be subdivided into two major groups: namely, scaffolding molecules (adhesion receptors, adaptor proteins, cytoskeletal proteins) and signaling/regulatory molecules (kinases, phosphatases, GTPases and their regulators, and proteases, among others) (Figure 2). However, some overlap exists between the groups, as certain multidomain proteins apparently display both docking and signaling functions (e.g., FAK [Carragher et al., 2003]). The assembly of the adhesome network is believed to be triggered by the binding of the extracellular domain of integrins to the ECM ligand.

This interaction induces conformational changes that separate the α and β subunits, thus exposing binding sites for cytoplasmic proteins within integrins' cytoplasmic tails (Kim et al., 2003). So far, no direct connection between integrins and actin has been found; rather, the integrin-actin interaction is mediated by a group of ~30 adaptor proteins. Some (e.g., paxillin) bind directly to integrins (Liu et al., 1999), others (e.g., vinculin) bind directly to actin (Johnson and Craig, 1995), and still others (e.g., talin) bind to both actin and integrins (Horwitz et al., 1986; McCann and Craig, 1999). Secondary adaptors (e.g., p130Cas) also exist that can bind to other adaptors, reinforcing the adhesion protein network (Sakai et al., 1994; Barrett et al., 2012). These interconnections between the different adaptors, together with additional signaling molecules, form the "focal adhesion plaque" that is connected to the actin stress fibers. This interaction is actually mediated via short, tangential actin fibers, as recently indicated by cryoelectron tomography studies (Patla et al., 2010; see below for further details). The formin mDia1 seems to play a central role in regulating stress fiber formation, because its knockdown slows the elongation rate of the actin filaments and alters the morphology of the stress fibers (Hotulainen and Lappalainen, 2006). In addition, Ena/VASP proteins tether actin filaments at sites of active assembly, thereby supporting actin filament elongation (Breitsprecher et al., 2008).
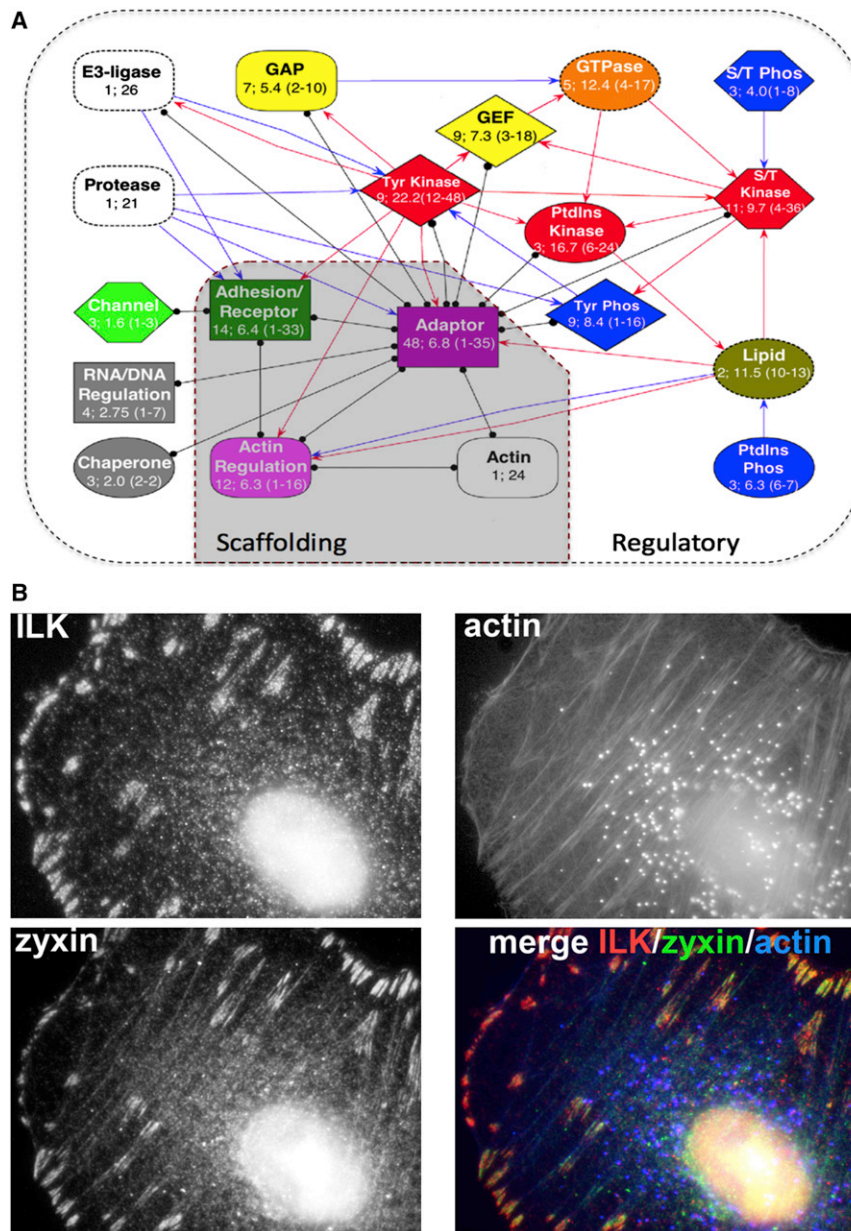
Recruitment of adaptor and signaling molecules to the developing adhesions appears to occur in a sequential manner, whereby certain proteins must be present at the adhesion site in order to recruit others (Zervas et al., 2011). A comprehensive understanding of the assembly hierarchy is still missing, yet the very early stages of adhesion assembly involve recruitment of talin to integrins, followed by paxillin and ILK, and subsequently vinculin, α-actinin, FAK, and, possibly, VASP (Zaidel-Bar et al., 2004). Actomyosin-generated forces subsequently applied to the adhesions may pull on several tension-sensitive

Figure 2. Molecular Complexity of Focal Adhesions

(A) Interactions between functional families of adhesome molecules. The various components of the adhesome form two major groups: scaffolding molecules and regulatory molecules. These groups have been further subdivided, according to the known biological activities of the different components. The dominant interactions between families (red arrows, activating interactions; blue arrows, inhibiting interactions; black lines, binding interactions) are shown (modified from Zaidel-Bar et al., 2007a). (B) To illustrate the complex molecular composition of focal adhesions, a HeLa cell was immunolabeled for three different adhesion components: ILK, zyxin, and actin. The merged image highlights the distinct localization of the different proteins within the same structures.

increasingly clear that they also act as sensing and signaling cellular "devices" capable of processing complex information induced either by the external micro- (and nano-) environment or, locally, by the adhesome complexes and the associated cytoskeleton (Chandrasekar et al., 2005; Choi et al., 2008; Na et al., 2008). It is noteworthy that unlike classical signaling receptors (e.g., receptor tyrosine kinases), integrins do not possess enzymatic activity; rather, their signaling properties are based on their capacity to recruit specific, "classical" adhesome signaling components to the adhesion site, thereby activating a wide variety of signaling networks (Avizienyte and Frame, 2005; Dupont et al., 2011).

Due to the great molecular complexity and diversity of the adhesome, as well as to their apparent cooperation with other transmembrane receptors (Worthington et al., 2011), integrins are able to sense and respond to different kinds of extracellular cues, including the chemical, physical, and topographical properties of the cell's microenvironment. The most critical chemical signal transmitted via integrins is the specific molecular composition of the ECM, including the relative contents of such molecules as fibronectin, vitronectin, collagen, and laminin (Humphries et al., 2006). To discriminate between these (and other) ECM ligands, integrins can form a wide variety of different α and β heterodimers. Mammals express 18 α subunits and eight β subunits and form up to 24 different functional heterodimers whose binding affinities differ, depending on the nature of the ECM ligand (Hynes, 2002). Cells can express and form different heterodimers at the same time, potentially increasing their ability to fine-tune the sensing of ECM composition. This process can be even more sophisticated, as the expression patterns of the different integrin subunits or the associated adhesome molecules (as well as of other associated transmembrane receptors) may

molecules (e.g., p130Cas, talin, vinculin [Sawada et al., 2006; del Rio et al., 2009; Grashoff et al., 2010]), thereby exposing additional binding sites and enabling further recruitment of proteins (e.g., zyxin) to the adhesion sites. Details of this process are still lacking; however, it is clear that it is driven by a finely tuned interplay between self-assembly and force-dependent assembly within adhesion-associated, multiprotein complexes.

Adhesion-Mediated Scaffolding and Signaling: Integrin Adhesions as Integrators of Environmental Sensing

Since the early days of cell adhesion research, integrin-mediated interactions were primarily viewed vis-à-vis their mechanical, tissue-scaffolding functions (Abercrombie and Dunn, 1975; Rees et al., 1977). In recent years, however, it has become

be modulated over time in response to changes in ECM composition, thereby altering the cellular response to the adhesive interactions (Yarwood and Woodgett, 2001; Kass et al., 2007).

Integrin adhesions can also sense the physical properties of the ECM, including its rigidity, ligand density, isotropy, and topography (Jiang et al., 2006; Théry et al., 2006; Cavalcanti-Adam et al., 2007). The specific molecular mechanisms whereby cells sense such physical cues are unclear, but a crucial step in these processes is the local response of integrin adhesions to mechanical forces applied to the adhesion sites, either by the matrix or by the contractile actomyosin machinery (Chen, 2008; Geiger et al., 2009). It was shown, for example, that changes in matrix rigidity can trigger the formation of adhesions with distinct morphologies, compositions, and signaling capabilities (Schlunck et al., 2008; Prager-Khoutorsky et al., 2011). Hence, on stiff substrates, cells typically produce larger and more stable adhesions whose molecular properties are clearly distinct from those formed on more compliant substrates (Prager-Khoutorsky et al., 2011). Furthermore, the different types of adhesions affect cytoskeletal organization and overall cell morphology, leading to the formation of elongated and polarized cells when on stiff substrates, compared to round, nonpolarized cells when on soft surfaces (Prager-Khoutorsky et al., 2011). Beyond these morphological changes, different ECM rigidities can lead to long-term physiological changes in the transcriptional program and in the regulation of protein expression and stability that can, eventually, affect cell behavior and fate (Engler et al., 2006; Dupont et al., 2011). Several pathways have been proposed that could explain how the application of force enables cells to sense rigidity, including conformational changes in proteins (del Rio et al., 2009), strengthening of integrin-ligand bonds (Choquet et al., 1997), and induction of specific phosphorylation events (Sawada et al., 2006) (for a detailed review, see Moore et al., 2010). However, the basic mechanism whereby physical cues (rigidity, force) are "translated" into chemical cues (e.g., phosphorylation, protein-protein interactions) is still not well characterized.

These examples of possible mechanisms responsible for adhesion-mediated signaling are just the tip of an exciting, poorly understood iceberg. The main challenges lying ahead involve not only characterization of the individual sensing processes of distinct physical properties, but also the integration of multiple environmental signals and the development of a coherent cellular response. These issues are beyond the scope of this article, although additional, still limited, information may be found in several recent review articles (Vogel and Sheetz, 2009; Geiger and Yamada, 2011; Bershadsky, 2012).

The Life Cycle of Integrin Adhesions: Formation, Transfiguration, and Dissociation

Live-cell microscopy studies have revealed four main stages in the "life cycle" of integrin adhesions; these include nascent adhesions, focal complexes, focal adhesions, and fibrillar adhesions. Nascent adhesions are submicron-sized structures that are usually "born" under the lamellipodium and are barely visible by means of ordinary fluorescence microscopy. It is believed that once the lamellipodium forms the first contact with the matrix, initial interaction of integrins with talin and kindlin takes place ("inside-out activation"), enhancing integrin activation and

stabilizing its grip on the ECM (Ma et al., 2008; Montanez et al., 2008). This is followed by integrin clustering and generation of the initial complexes: namely, nascent adhesions. This process seems to occur very rapidly, on a timescale of seconds (Yu et al., 2011), and involves only a small number of integrin molecules. Moreover, assembly of nascent adhesions is seemingly independent of myosin II activity but does induce actin polymerization (Yu et al., 2011). This finding suggests an essential role for actin-nucleating protein(s) in these early adhesions; however, to date, such information is still lacking.

The subsequent pull of myosin on these clusters reinforces the strength of the adhesions (Roca-Cusachs et al., 2009) and is believed to lead to the recruitment of additional adhesome proteins to the adhesion sites, leading to growth of these clusters into the somewhat larger focal complexes. Nascent adhesions and focal complexes display similar molecular compositions and seem to differ mainly in their myosin dependence and size (Choi et al., 2008). Focal complexes are small (~1 μm diameter), punctate, and short-lived structures (a lifetime of ~1–2 min) that are located close to the edge of the lamellipodium (Choi et al., 2008). These transient entities either disassemble by an as-yet unknown mechanism or rapidly grow centripetally and evolve into larger, elongated focal adhesions. The growth process clearly depends on forces generated by actomyosin-based stress fibers (Riveline et al., 2001; Choi et al., 2008), but it also requires that the stress fibers serve as physical contractile anchors (Oakes et al., 2012).

Focal adhesions are considerably larger and more elongated (commonly ~1 μm wide, 3–5 μm long) than focal complexes, with typical lifetimes of up to several tens of minutes (Zaidel-Bar et al., 2007b). The most prominent protein marker for mature focal adhesions is zyxin, as it is recruited to the adhesions at later stages in their development and does not appear in nascent adhesions and focal complexes (Zaidel-Bar et al., 2003). As they mature, the growing focal adhesions define a new boundary between the lamellipodium and the "lamella proper" (Alexandrova et al., 2008; Burnette et al., 2011) and are believed to play a key role in supporting the forward protrusion of the leading edge of migrating cells (Ponti et al., 2004). The disassembly of focal adhesions often occurs at the rear of the cell when the "tail" of the cell is retracting. This process involves microtubule-mediated destabilization of the adhesions and plays an important role during persistent cell migration (Kaverina et al., 1999).

Another mechanism leading to loss of focal adhesions involves the transformation of these adhesion sites into another type of integrin-mediated contact: fibrillar adhesions. These structures are considerably more elongated than most focal adhesions and are located (mostly in fibroblasts) around the cell center, where they are associated with fibronectin fibrils (Pankov et al., 2000). In fact, a characteristic property of fibrillar adhesions is their capacity to remodel the ECM and induce fibrillogenesis (Pankov et al., 2000). The transformation requires mechanical force, but the maintenance of these adhesions is far less sensitive to inhibition of actomyosin contractility compared to classical focal adhesions (Zaidel-Bar et al., 2007b).

The transformation of one form of adhesion into another is tightly regulated by the cellular signaling system, primarily by small GTPases, including Rac1, cdc42, and RhoA (Jaffe and
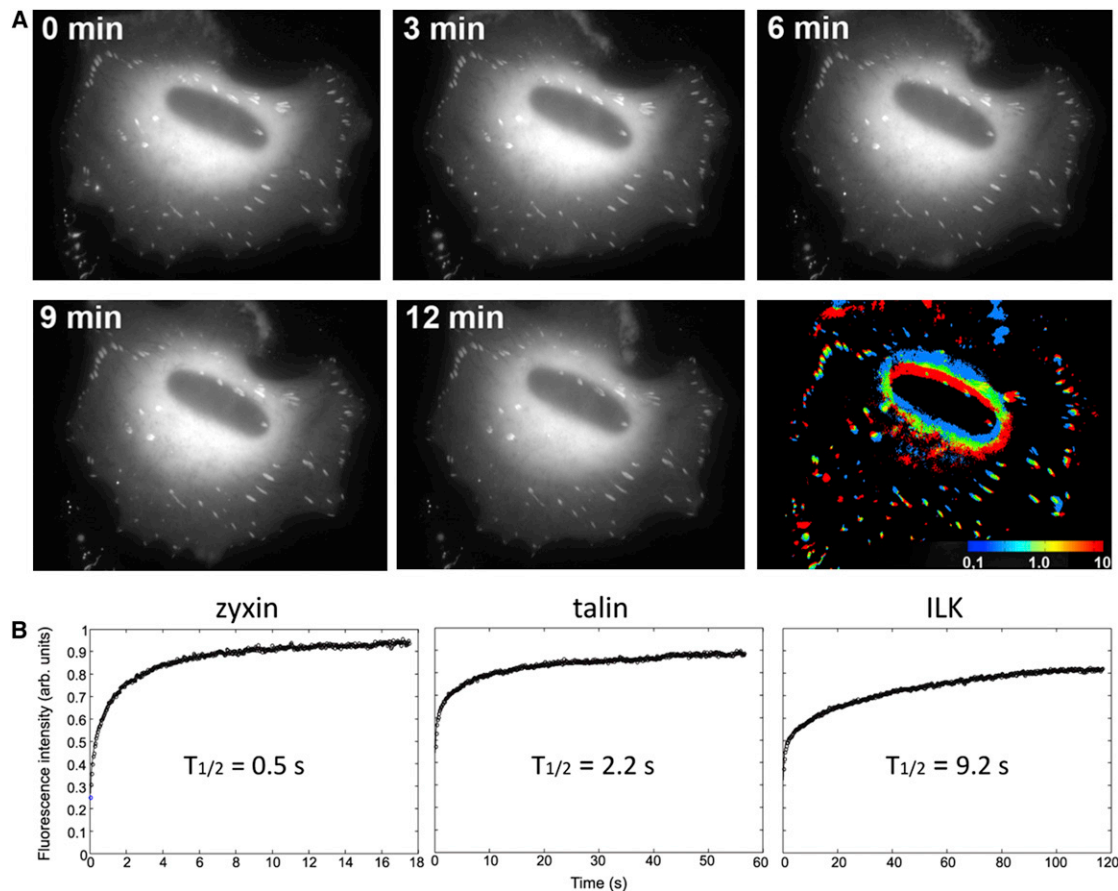
Figure 3. Molecular and Structural Kinetics of Focal Adhesions
(A) Structural dynamics of focal adhesions. A HeLa cell expressing GFP-vinculin was recorded for 12 min by time-lapse fluorescence microscopy. The resulting images were analyzed by temporal ratio imaging (bottom right image), enabling comparison of the same structures at different time points. A red shift denotes structures with decreased intensity; a blue shift denotes an increase in intensity; green and yellow hues mark unchanged pixels. (B) Different focal adhesion proteins display varying turnover rates. Normalized averaged curves of FRAP experiments performed in HeLa cells on different GFP-tagged adhesion-related proteins are shown. In each case, an average of 20–30 FRAP curves is displayed. Note the different timescales of the measurements, as well as the half-time to full recovery ($T_{1/2}$) values.

Hall, 2005). Rac1 and Cdc42 have long been implicated in the formation of focal complexes and actin polymerization, whereas RhoA is associated with focal adhesion maturation (Nobes and Hall, 1995; Rottner et al., 1999). Notably, Cdc42 can activate Rac1 (Nobes and Hall, 1995), and Rac1 and RhoA suppress each other's activity (Yamaguchi et al., 2001; Nimnual et al., 2003). Thus, as focal adhesions grow and mature, Cdc42 and Rac1 activities are suppressed, and RhoA activates myosin pulling on actin fibers by phosphorylating myosin light-chain (MLC) via Rho kinase (ROCK). Transition of focal adhesions into fibrillar adhesions requires further RhoA-mediated contractility and the centripetal translocation of ligated $\alpha_5\beta_1$ integrins from the focal adhesions, accompanied by the stretching of soluble fibronectin dimers (Pankov et al., 2000). A recent study using FRET biosensors indicated that RhoA is activated at the cell edge about 40 s prior to Cdc42 and Rac1, which are activated 2 μm behind RhoA (Machacek et al., 2009). This finding indicates that RhoA may play a role in the early formation of nascent adhesions, and, subsequently, the coordinated action of Rac1 and Cdc42 leads to growth into slightly larger focal

complexes. For additional reading on the transitions between the different forms of adhesions, see recent reviews (Wolfenson et al., 2009a; Vicente-Manzanares and Horwitz, 2011; Bershadsky, 2012).

Structural and Dynamic Characterization of Integrin Adhesions: Stability versus Turnover
One of the intriguing aspects of integrin adhesions is that despite their capacity to persist for relatively long periods of time with minimal change (Figure 3A), they are able to rapidly undergo radical alterations (e.g., disassembly upon reduction of actomyosin contractility). To address the mechanism underlying such variations in focal adhesions, several studies have focused on the molecular kinetics of integrin adhesions, mainly using fluorescence recovery after photobleaching (FRAP) or fluorescence correlation approaches. These studies revealed that even in apparently stable focal adhesions, there is a constant turnover of proteins on timescales of milliseconds to seconds (Wolfenson et al., 2009a). Moreover, within the adhesions themselves, different adhesome proteins (mainly adaptors)

display vastly different turnover rates (Figure 3B). These rates depend on the state of the adhesion (e.g., growing, at steady state, disassembling, or "sliding") (Digman et al., 2008), and most likely reflect differences in the available binding sites for each molecule.

On average, each molecule within the adhesome has ∼8–10 potential binding partners (Zaidel-Bar et al., 2007a). Although this number does not necessarily reflect the actual situation in individual cells at any given time point, it demonstrates the molecular complexity of integrin adhesions and the difficulty in determining the factors that regulate adhesion dynamics at the molecular level. In general, it appears likely that over time, as adhesions develop, more and more binding sites are exposed to cytoplasmic proteins at or near existing adhesion sites, leading to an apparently more avid interaction of these proteins with the adhesions. Several examples support this hypothesis, including force-induced exposure of cryptic vinculin binding sites within the talin rod (del Rio et al., 2009), very rapid accumulation of vinculin into growing adhesions following application of force (Riveline et al., 2001), and an increase in focal adhesion area upon expression of vinculin mutants with exposed binding sites (Humphries et al., 2007), among others.

Examination of the dynamic properties of diverse focal adhesion-associated proteins points to considerable variability. In stable and mature adhesions, most plaque proteins display both dynamic and stable subpopulations of components (the latter is demonstrated by a 20%–40% immobile fraction in the FRAP studies) (Wolfenson et al., 2009b). Furthermore, stable adhesions also display varying molecular exchange rates, depending on their location—e.g., distal or proximal regions—within individual adhesions (Digman et al., 2008; Wolfenson et al., 2009b). This phenomenon has been implicated with differential force distribution within focal adhesions (Shemesh et al., 2005; Besser and Safran, 2006) or with different phosphorylation states of specific proteins (e.g., paxillin; Zaidel-Bar et al., 2007a)—two phenomena that are possibly linked. In fact, mechanical forces are major regulators of the kinetics of plaque proteins, as demonstrated by studies utilizing inhibitors of actomyosin contractility (Pasapera et al., 2010; Wolfenson et al., 2011). Interestingly, different plaque proteins vary in their response to force reduction, as some (e.g., vinculin) display higher FRAP rates and mobile fractions while others (e.g., paxillin) display lower rates, compared to their values prior to myosin inhibition (Wolfenson et al., 2011). This phenomenon is not entirely understood, but additional studies indicate that the stability of focal adhesions is regulated by the binding and unbinding rates of the exchanging proteins with the adhesions: once there is a reduction in the degree of force applied to the adhesion, these rates change such that there is a lower binding rate and a higher unbinding rate (H.W. and B.G., unpublished data).

Taken together, these dynamic results suggest that focal adhesions are not uniform entities; rather, they contain different subpopulations of plaque proteins within them. This finding is particularly interesting in view of recent nanoscale structural data generated using cryoelectron tomography (Medalia and Geiger, 2010; Patla et al., 2010) and the superresolution fluorescence technique, iPALM (Kanchanawong et al., 2010). The EM studies revealed that the transmembrane interactions at focal adhesions are mediated via discrete particles of ∼25 nm in size that are attached to both the inner surface of the plasma membrane and the actin fibers. These results not only suggest that focal adhesions contain multiple "hot spots" of cytoskeletal anchorage (rather than a uniform protein mesh), but they also raise the possibility that differences in composition and molecular turnover may exist between the various particles within individual adhesions (Wolfenson et al., 2009b). In the superresolution iPALM studies (Kanchanawong et al., 2010), the z axis distribution of focal adhesion proteins was investigated, confirming that focal adhesions are laminated structures and that the integrins and actin inside focal adhesions are vertically bridged by a layer of plaque proteins composed of several subregions. These results imply a hierarchical distribution of different plaque proteins within focal adhesions, whereby integrin-binding proteins are located close to the plasma membrane and actin-binding proteins are located close to the actin filaments, ∼40 nm above the integrins. Such superresolution techniques, capable of reaching xy resolution of a few tens of nanometers, hold promise for future research in determining the nanoscale composition of integrin adhesions and may help to determine the nature of the nanostructures observed in the cryo-EM studies. Moreover, by using sophisticated fluorescent probes and constructs such as FRET sensors (Grashoff et al., 2010) or double-tagged molecules (Margadant et al., 2011) in combination with superresolution microscopy, it may be possible to clarify the molecular mechanisms underlying the regulation of adhesion dynamics.

The Structure and Function of Integrin Adhesions In Vivo

Widespread interest in cell adhesion, originating in the 1970s, was largely initiated and motivated by developmental biologists (Hay, 1981; Sanes, 1983; Thiery et al., 1985). As a consequence, most of the research on cell-cell and cell-matrix adhesions was initially directed toward understanding the roles of adhesion processes during embryogenesis and organogenesis. Nevertheless, shortly thereafter, cultured cells growing in a monolayer became the primary models for studying integrin adhesions. The reasons for this choice included the convenience of working with such cells, the availability of excellent imaging systems and biophysical tools for studying the adhesion structures, the amenability of such cellular systems to genetic perturbation (e.g., using small interfering RNA technology) or live-cell imaging (e.g., using intrinsically fluorescent proteins), and the possibility of engineering specific adhesive surfaces that could stimulate cell growth and differentiation.

Naturally, the question of whether "classical focal adhesions" viewed in experiments on two-dimensional (2D) surfaces indeed constitute suitable models for integrin adhesions was frequently asked over the years, as these structures are certainly much harder to detect in vivo (Burridge et al., 1988). It is certainly possible that, for certain cell types, a hard, flat surface imposes an artificial phenotype, resulting in adhesion structures that do not exist in vivo in the same cells. For example, focal adhesion proteins are diffusely localized in salivary gland epithelial cells in vivo but are concentrated in focal adhesions when the cells are plated on 2D surfaces (Sequeira et al., 2012). On the other hand, many indications from other cell types imply that similar adhesions do exist in vivo. A classic example is that of smooth muscle cells, in which most of the early studies on cell-matrix

adhesions were performed (Burridge et al., 1988). These cells produce large, stable adhesions with the ECM that are required for cell stabilization during generation of force within the tissue. The molecular characteristics of these adhesions are similar to focal adhesions that form in vitro.

While there is probably no definitive answer to the concerns described above, certain obvious differences between matrix adhesions in the body and those on a coverslip motivated researchers to engineer "bio-inspired" adhesive surfaces, which more closely resemble physiological adhesion conditions. These include the formation of more compliant matrices, the use of three-dimensional (3D) matrices, and the modulation of the adhesive epitopes and their density, among others (reviewed in Geiger et al., 2009). Importantly, through the use of such surfaces, it immediately became apparent that the adhesion structures are altered in various ways. For instance, cells grown on 2D soft gels form focal adhesions that are much smaller and more unstable than those formed on stiffer surfaces (Pelham and Wang, 1997). The molecular mechanisms underlying this observation are still unclear, but researchers have begun to address this issue (Prager-Khoutorsky et al., 2011; Ghassemi et al., 2012; Trappmann et al., 2012). Recently, this phenomenon was also observed in studies of cells grown in 3D collagen matrices (Fraley et al., 2010): cells located close to the edge of the collagen gel formed focal adhesions at their lamellipodia, whereas in cells embedded more deeply within the matrix (where they presumably sense lower tensile forces), focal adhesion proteins were distributed diffusely throughout the cytoplasm, rather than in adhesions. Notably, these results were challenged by the claim that reducing the level of background fluorescence reveals focal adhesions even in deeply embedded cells (Kubow and Horwitz, 2011). The differences between the two groups' results are not clear, and they may arise from differences in preparation of the 3D matrices. However, these seemingly contradictory results can give us insights into the reasons for the difficulties in detecting focal adhesions in vivo: cells that are located at less-rigid locations may produce adhesions so small that they cannot be visualized using traditional fluorescence microscopy. Importantly, in the aforementioned study, even when focal adhesions were not noticeable in deeply embedded cells, focal adhesion proteins did play a major role in cellular motility in the 3D environment (Fraley et al., 2010). Certainly, studies of focal adhesions in 3D cultures are only in their initial stages, and the proper technical conditions for these studies are still being resolved. These issues should also be addressed in the future using novel superresolution fluorescence microscopy techniques.

Other bio-inspired surfaces that were produced in recent years to study integrin adhesions include modulations of the distances between integrin ligands (Cavalcanti-Adam et al., 2007; Schvartzman et al., 2011), surfaces with several types of ligands (Holst et al., 2010), and micropatterned surfaces with specific ligand organization (Théry et al., 2006). All of these specialized surfaces altered the adhesions in various ways, lending support to the view that integrins are, indeed, classical surface sensors.

In view of these (and other) results, it is clear that tissue culture systems are not only very relevant, but in fact also constitute very powerful models for the study of matrix adhesions. Because integrin adhesions function to integrate various types of environmental signals, dissecting these signals in vivo, as well as the molecular mechanisms that guide them, is extremely difficult, let alone at high temporal and spatial resolution. In this sense, "bio-inspired" artificial surfaces can yield new, exciting information as to the role of matrix dimensionality, compliance, and anisotropy, for example, in scaling and modulating the adhesions, affecting both their structure and signaling activities. The advances in surface chemistry and surface engineering during the last decade provide novel, powerful tools to study these processes in unprecedented detail and in a highly quantitative manner.

Functions of Integrin Adhesions in Development

The critical role of integrins in mediating cell adhesion to the ECM has attracted the attention of many developmental biologists wishing to clarify their role in embryogenesis and organogenesis. In a series of studies beginning in the early 1990s, researchers systematically knocked out the different α- and β-integrin subunits in mice to study their roles at various developmental stages (reviewed in Bouvard et al., 2001). These knockouts resulted in various developmental phenotypes, from minor apparent effects to early embryonic lethality. For instance, knockout of $β_1$, the most abundantly expressed integrin subunit, capable of forming up to 12 types of dimers with different α subunits (Hynes, 2002), led to very early lethality due to failure of the embryo to develop the inner cell mass (Fässler and Meyer, 1995). Mice that lacked $β_5$-integrin showed no apparent change in phenotype at first (Huang et al., 2000), but further investigations have shown defects in the adult mice, such as reduced vascular permeability in response to VEGF (Eliceiri et al., 2002) or defective retinal phagocytosis (Nandrot et al., 2004), among others. Other types of integrins have more distinct roles in specialized biological systems such as immune cells; as a result, their ablation does not induce early lethality, but instead leads to impaired immune function only under specific circumstances. For example, in the absence of $α_L$-integrin (more commonly known as lymphocyte function-associated antigen, or LFA-1), which is expressed on the surface of leukocytes, mice are viable and display a normal immune response against systemic viral infections; however, their response to injected immunogenic tumor cells is impaired, and they cannot reject the tumors (Schmits et al., 1996). Other developmental studies involved knockouts or mutations of central adhesion plaque proteins, as well as of ECM genes (e.g., fibronectin, vitronectin, or collagen). These, too, resulted in various phenotypes, including lethality or impaired development of critical organs such as the cardiovascular system, kidney, brain, and muscle (Gustafsson and Fässler, 2000; Bökel and Brown, 2002).

Studies that involved phenotypic analyses of mutated *Drosophila melanogaster* have proven to be very valuable in elucidating not only developmental processes but also the fundamental components that play roles in regulating integrin-mediated adhesion. The vast knowledge of *Drosophila* development, combined with the ease of mutating and the low number of adhesion-related genes, has allowed screening for mutants with similar phenotypes and, thus, the construction of maps of pathways downstream of integrins. For example, as early as 1918, a *Drosophila* mutant was identified that had blisters in its wings (Weinstein, 1918); later it was shown that this gene (named *inflated*) encodes the integrin αPS2 subunit (Bogaert et al., 1987),

which is required for the proper connection of the two opposing epithelial sheets that make up the adult wing (Brower and Jaffe, 1989). In subsequent genetic screens, researchers identified several mutants that had similar phenotypes to the *inflated* mutant, as well as other defects in wing and muscle development (Volk et al., 1990; Wilcox, 1990). Among others, some of the central genes that were identified as critical were the orthologs for talin (Brown et al., 2002), ILK (Zervas et al., 2001), PINCH (Clark et al., 2003), and, later, parvin (Vakaloglou et al., 2012). The latter three are referred to as the IPP complex, which is apparently formed in the cytoplasm and then interacts with β-integrins to link them to the actin cytoskeleton. Interestingly, in muscles, the stability and localization of the IPP complex depends on ILK alone, but in the wing epithelium all three proteins are mutually dependent on each other (Vakaloglou et al., 2012). This suggests that the proper regulation of integrin adhesions may differ between different tissues during development.

The phenotypic analysis of different mutants also supports the view that a hierarchy of function exists between the proteins involved in adhesion regulation. Thus, integrin is activated by talin, which subsequently recruits the IPP triplet (of which ILK's scaffolding function seems to be central), as well as paxillin and tensin. In recent years, a new level of complexity arose when the kindlin family (which was first identified in *Caenorhabditis elegans* [Rogalski et al., 2000]) was shown to coregulate integrin activation together with talin. Moreover, kindlins, too, can act as scaffolding proteins, recruiting adhesion-related proteins (including ILK) to the adhesion sites (Karaköse et al., 2010). Kindlin deletion in mice results in major adhesion-related defects, depending on the expression pattern of the specific kindlin isoform—from severe bleeding (kindlin 3) (Moser et al., 2008), to skin atrophy and a detached colon epithelium (kindlin 1) (Siegel et al., 2003; Ussar et al., 2008), or death at implantation (kindlin 2) (Montanez et al., 2008)—all due to defective integrin function.

Recent improvements in fluorescence microscopy have enabled time-lapse imaging of single cells or groups of cells in living organisms. These advances paved the way toward in vivo studies of integrin adhesions during embryonic development. Thus, it was shown that disrupting or enhancing cell-ECM adhesions in *Drosophila* results in altered cytoskeletal forces and, as a consequence, altered tissue elongation and shape (He et al., 2010). It was also shown that integrin signaling proteins mediate actomyosin-generated cues that lead to increased tissue stiffness and proliferation (Samuel et al., 2011). In a recent study, FRAP measurements were performed on *Drosophila* embryos to study the effect of tensile force on integrin adhesion turnover in vivo (Pines et al., 2012). Using temperature-sensitive mutations that caused muscle contraction, Pines et al. showed that, under increased tension, the rate of integrin turnover (measured as the ratio between integrin endocytosis and delivery to the membrane) is low and the adhesions are stable, whereas under reduced tension integrin turnover is high and the adhesions are less stable. These results corroborate the in vitro findings on the role of forces in regulating adhesion dynamics (see above), and also provide a link between forces, adhesion stability, and tissue development.

Taken together, the above-mentioned studies demonstrated the diverse roles of integrin adhesions in developing tissues and organs, including scaffolding processes involved in tissue and organ morphogenesis, and environmental sensing processes whereby integrin-mediated interactions activate specific signaling networks. They also highlighted the importance of using model organisms in which the number of integrin-related adhesion components is smaller, thus enabling one to distinguish between critical and basic components that emerged early in evolution, and therefore exist in *Drosophila* and *C. elegans*, and more "sophisticated" components, which might be critical in more specific processes.

Future Prospects and Open Questions Relevant to the Involvement of Integrin Adhesions in Development

The apparent complexity and diversity of the adhesome offer major challenges in analyzing the structure-function relationship of integrin adhesions. The huge diversity of the adhesome provides important versatility whereby the cellular adhesion machinery can respond, locally, to the rich variety of extracellular matrices it encounters. Moreover, the dynamic nature of these adhesions enables them to sense and respond to temporal processes such as matrix remodeling and transcellular stress. The mechanisms underlying the complex crosstalk between the microenvironment and the integrin adhesome network remain poorly characterized, and novel experimental approaches are needed to clarify, at the molecular level, processes such as matrix-induced assembly of the adhesion sites and matrix mechanosensing, both of which take place in integrin adhesions. Encouraging results from a variety of cell biological models, using novel microscopic technologies, powerful computational approaches, and nano-engineered adhesive surfaces, offer promising insights into adhesion biology that might prove relevant to important processes taking place within the developing organism. Among these novel technologies, we would highlight, in particular, the in vivo characterization of the dynamics of adhesions, using multiple, intrinsically fluorescent adhesome components, and the accurate measurement of their molecular exchange rates and turnover. Emerging cutting-edge technologies such as superresolution microscopy and cryoelectron tomography (and their possible use in tandem) can shed further light on the inner molecular architecture of adhesion sites at the nanoscale level. The major future challenge lies in the integration of this dynamic, complex, and multidimensional information with a mechanistic understanding of adhesion and adhesion-mediated signaling processes.

REFERENCES

Abercrombie, M., and Dunn, G.A. (1975). Adhesions of fibroblasts to substratum during contact inhibition observed by interference reflection microscopy. Exp. Cell Res. *92*, 57–62.

Abercrombie, M., and Heaysman, J.E. (1954). Invasiveness of sarcoma cells. Nature *174*, 697–698.

Abercrombie, M., Heaysman, J.E., and Pegrum, S.M. (1971). The locomotion of fibroblasts in culture. IV. Electron microscopy of the leading lamella. Exp. Cell Res. *67*, 359–367.

Alexandrova, A.Y., Arnold, K., Schaub, S., Vasiliev, J.M., Meister, J.J., Bershadsky, A.D., and Verkhovsky, A.B. (2008). Comparative dynamics of retrograde actin flow and focal adhesions: formation of nascent adhesions triggers transition from fast to slow flow. PLoS ONE *3*, e3234.

Algard, F.T. (1953). Morphology and migratory behavior of embryonic pigment cells studied by phase microscopy. J. Exp. Zool. *123*, 499–521.

Avizienyte, E., and Frame, M.C. (2005). Src and FAK signalling controls adhesion fate and the epithelial-to-mesenchymal transition. Curr. Opin. Cell Biol. *17*, 542–547.

Barrett, A., Pellet-Many, C., Zachary, I.C., Evans, I.M., and Frankel, P. (2012). p130Cas: A key signalling node in health and disease. Cell. Signal. *25*, 766–777.

Bershadsky, A. (2012). Cell-matrix adhesions. Cellular structures in mechanosensing and cell motility, in MBInfo. https://sites.google.com/a/mechanobio.info/mbinfo/Home/Dynamic-Structures-in-Mechanosensing/cell-matrix-adhesions.

Besser, A., and Safran, S.A. (2006). Force-induced adsorption and anisotropic growth of focal adhesions. Biophys. J. *90*, 3469–3484.

Bogaert, T., Brown, N., and Wilcox, M. (1987). The Drosophila PS2 antigen is an invertebrate integrin that, like the fibronectin receptor, becomes localized to muscle attachments. Cell *51*, 929–940.

Bökel, C., and Brown, N.H. (2002). Integrins in development: moving on, responding to, and sticking to the extracellular matrix. Dev. Cell *3*, 311–321.

Bouvard, D., Brakebusch, C., Gustafsson, E., Aszódi, A., Bengtsson, T., Berna, A., and Fässler, R. (2001). Functional consequences of integrin gene mutations in mice. Circ. Res. *89*, 211–223.

Breitsprecher, D., Kiesewetter, A.K., Linkner, J., Urbanke, C., Resch, G.P., Small, J.V., and Faix, J. (2008). Clustering of VASP actively drives processive, WH2 domain-mediated actin filament elongation. EMBO J. *27*, 2943–2954.

Brower, D.L., and Jaffe, S.M. (1989). Requirement for integrins during Drosophila wing development. Nature *342*, 285–287.

Brown, N.H., Gregory, S.L., Rickoll, W.L., Fessler, L.I., Prout, M., White, R.A., and Fristrom, J.W. (2002). Talin is essential for integrin function in Drosophila. Dev. Cell *3*, 569–579.

Burnette, D.T., Manley, S., Sengupta, P., Sougrat, R., Davidson, M.W., Kachar, B., and Lippincott-Schwartz, J. (2011). A role for actin arcs in the leading-edge advance of migrating cells. Nat. Cell Biol. *13*, 371–381.

Burridge, K., and Feramisco, J.R. (1980). Microinjection and localization of a 130K protein in living fibroblasts: a relationship to actin and fibronectin. Cell *19*, 587–595.

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

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.

Cavalcanti-Adam, E.A., Volberg, T., Micoulet, A., Kessler, H., Geiger, B., and Spatz, J.P. (2007). Cell spreading and focal adhesion dynamics are regulated by spacing of integrin ligands. Biophys. J. *92*, 2964–2974.

Chambers, R., and Fell, H.B. (1931). Micro-operations on cells in tissue cultures. Proc. Roy. Soc. Ser. B *109*, 380–403.

Chandrasekar, I., Stradal, T.E., Holt, M.R., Entschladen, F., Jockusch, B.M., and Ziegler, W.H. (2005). Vinculin acts as a sensor in lipid regulation of adhesion-site turnover. J. Cell Sci. *118*, 1461–1472.

Chen, C.S. (2008). Mechanotransduction - a field pulling together? J. Cell Sci. *121*, 3285–3292.

Choi, C.K., Vicente-Manzanares, M., Zareno, J., Whitmore, L.A., Mogilner, A., and Horwitz, A.R. (2008). Actin and alpha-actinin orchestrate the assembly and maturation of nascent adhesions in a myosin II motor-independent manner. Nat. Cell Biol. *10*, 1039–1050.

Choquet, D., Felsenfeld, D.P., and Sheetz, M.P. (1997). Extracellular matrix rigidity causes strengthening of integrin-cytoskeleton linkages. Cell *88*, 39–48.

Clark, K.A., McGrail, M., and Beckerle, M.C. (2003). Analysis of PINCH function in Drosophila demonstrates its requirement in integrin-dependent cellular processes. Development *130*, 2611–2621.

Curtis, A.S. (1964). The mechanism of adhesion of cells to glass. A study by interference reflection microscopy. J. Cell Biol. *20*, 199–215.

del Rio, A., Perez-Jimenez, R., Liu, R., Roca-Cusachs, P., Fernandez, J.M., and Sheetz, M.P. (2009). Stretching single talin rod molecules activates vinculin binding. Science *323*, 638–641.

Digman, M.A., Brown, C.M., Horwitz, A.R., Mantulin, W.W., and Gratton, E. (2008). Paxillin dynamics measured during adhesion assembly and disassembly by correlation spectroscopy. Biophys. J. *94*, 2819–2831.

Dupont, S., Morsut, L., Aragona, M., Enzo, E., Giulitti, S., Cordenonsi, M., Zanconato, F., Le Digabel, J., Forcato, M., Bicciato, S., et al. (2011). Role of YAP/TAZ in mechanotransduction. Nature *474*, 179–183.

Eliceiri, B.P., Puente, X.S., Hood, J.D., Stupack, D.G., Schlaepfer, D.D., Huang, X.Z., Sheppard, D., and Cheresh, D.A. (2002). Src-mediated coupling of focal adhesion kinase to integrin alpha(v)beta5 in vascular endothelial growth factor signaling. J. Cell Biol. *157*, 149–160.

Engler, A.J., Sen, S., Sweeney, H.L., and Discher, D.E. (2006). Matrix elasticity directs stem cell lineage specification. Cell *126*, 677–689.

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

Fraley, S.I., Feng, Y., Krishnamurthy, R., Kim, D.H., Celedon, A., Longmore, G.D., and Wirtz, D. (2010). A distinctive role for focal adhesion proteins in three-dimensional cell motility. Nat. Cell Biol. *12*, 598–604.

Geiger, B. (1979). A 130K protein from chicken gizzard: its localization at the termini of microfilament bundles in cultured chicken cells. Cell *18*, 193–205.

Geiger, B., and Yamada, K.M. (2011). Molecular architecture and function of matrix adhesions. Cold Spring Harb. Perspect. Biol. *3*, 201–221.

Geiger, T., and Zaidel-Bar, R. (2012). Opening the floodgates: proteomics and the integrin adhesome. Curr. Opin. Cell Biol. *24*, 562–568.

Geiger, B., Spatz, J.P., and Bershadsky, A.D. (2009). Environmental sensing through focal adhesions. Nat. Rev. Mol. Cell Biol. *10*, 21–33.

Ghassemi, S., Meacci, G., Liu, S., Gondarenko, A.A., Mathur, A., Roca-Cusachs, P., Sheetz, M.P., and Hone, J. (2012). Cells test substrate rigidity by local contractions on submicrometer pillars. Proc. Natl. Acad. Sci. USA *109*, 5328–5333.

Goodrich, H.B. (1924). Cell behavior in tissue cultures. Biol. Bull. *46*, 252–262.

Grashoff, C., Hoffman, B.D., Brenner, M.D., Zhou, R., Parsons, M., Yang, M.T., McLean, M.A., Sligar, S.G., Chen, C.S., Ha, T., and Schwartz, M.A. (2010). Measuring mechanical tension across vinculin reveals regulation of focal adhesion dynamics. Nature *466*, 263–266.

Gustafsson, E., and Fässler, R. (2000). Insights into extracellular matrix functions from mutant mouse models. Exp. Cell Res. *261*, 52–68.

Harris, A. (1973). Location of cellular adhesions to solid substrata. Dev. Biol. *35*, 97–114.

Harrison, R.G. (1911). On the stereotropism of embryonic cells. Science *34*, 279–281.

Hay, E.D. (1981). Extracellular matrix. J. Cell Biol. *91*, 205s–223s.

He, L., Wang, X., Tang, H.L., and Montell, D.J. (2010). Tissue elongation requires oscillating contractions of a basal actomyosin network. Nat. Cell Biol. *12*, 1133–1142.

Heath, J.P., and Dunn, G.A. (1978). Cell to substratum contacts of chick fibroblasts and their relation to the microfilament system. A correlated

interference-reflexion and high-voltage electron-microscope study. J. Cell Sci. *29*, 197–212.

Heggeness, M.H., Ash, J.F., and Singer, S.J. (1978). Transmembrane linkage of fibronectin to intracellular actin-containing filaments in cultured human fibroblasts. Ann. N Y Acad. Sci. *312*, 414–417.

Holst, J., Watson, S., Lord, M.S., Eamegdool, S.S., Bax, D.V., Nivison-Smith, L.B., Kondyurin, A., Ma, L., Oberhauser, A.F., Weiss, A.S., and Rasko, J.E. (2010). Substrate elasticity provides mechanical signals for the expansion of hemopoietic stem and progenitor cells. Nat. Biotechnol. *28*, 1123–1128.

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.

Hotulainen, P., and Lappalainen, P. (2006). Stress fibers are generated by two distinct actin assembly mechanisms in motile cells. J. Cell Biol. *173*, 383–394.

Huang, X., Griffiths, M., Wu, J., Farese, R.V., Jr., and Sheppard, D. (2000). Normal development, wound healing, and adenovirus susceptibility in beta5-deficient mice. Mol. Cell. Biol. *20*, 755–759.

Humphries, J.D., Byron, A., and Humphries, M.J. (2006). Integrin ligands at a glance. J. Cell Sci. *119*, 3901–3903.

Humphries, J.D., Wang, P., Streuli, C., Geiger, B., Humphries, M.J., and Ballestrem, C. (2007). Vinculin controls focal adhesion formation by direct interactions with talin and actin. J. Cell Biol. *179*, 1043–1057.

Humphries, J.D., Byron, A., Bass, M.D., Craig, S.E., Pinney, J.W., Knight, D., and Humphries, M.J. (2009). Proteomic analysis of integrin-associated complexes identifies RCC2 as a dual regulator of Rac1 and Arf6. Sci. Signal. *2*, ra51.

Hynes, R.O. (1987). Integrins: a family of cell surface receptors. Cell *48*, 549–554.

Hynes, R.O. (2002). Integrins: bidirectional, allosteric signaling machines. Cell *110*, 673–687.

Hynes, R.O., and Destree, A.T. (1978). Relationships between fibronectin (LETS protein) and actin. Cell *15*, 875–886.

Izzard, C.S., and Lochner, L.R. (1976). Cell-to-substrate contacts in living fibroblasts: an interference reflexion study with an evaluation of the technique. J. Cell Sci. *21*, 129–159.

Jaffe, A.B., and Hall, A. (2005). Rho GTPases: biochemistry and biology. Annu. Rev. Cell Dev. Biol. *21*, 247–269.

Jiang, G., Huang, A.H., Cai, Y., Tanase, M., and Sheetz, M.P. (2006). Rigidity sensing at the leading edge through alphavbeta3 integrins and RPTPalpha. Biophys. J. *90*, 1804–1809.

Johnson, R.P., and Craig, S.W. (1995). F-actin binding site masked by the intramolecular association of vinculin head and tail domains. Nature *373*, 261–264.

Kanchanawong, P., Shtengel, G., Pasapera, A.M., Ramko, E.B., Davidson, M.W., Hess, H.F., and Waterman, C.M. (2010). Nanoscale architecture of integrin-based cell adhesions. Nature *468*, 580–584.

Karaköse, E., Schiller, H.B., and Fässler, R. (2010). The kindlins at a glance. J. Cell Sci. *123*, 2353–2356.

Kass, L., Erler, J.T., Dembo, M., and Weaver, V.M. (2007). Mammary epithelial cell: influence of extracellular matrix composition and organization during development and tumorigenesis. Int. J. Biochem. Cell Biol. *39*, 1987–1994.

Kaverina, I., Krylyshkina, O., and Small, J.V. (1999). Microtubule targeting of substrate contacts promotes their relaxation and dissociation. J. Cell Biol. *146*, 1033–1044.

Kim, M., Carman, C.V., and Springer, T.A. (2003). Bidirectional transmembrane signaling by cytoplasmic domain separation in integrins. Science *301*, 1720–1725.

Kreis, T.E., Winterhalter, K.H., and Birchmeier, W. (1979). In vivo distribution and turnover of fluorescently labeled actin microinjected into human fibroblasts. Proc. Natl. Acad. Sci. USA *76*, 3814–3818.

Kubow, K.E., and Horwitz, A.R. (2011). Reducing background fluorescence reveals adhesions in 3D matrices. Nat. Cell Biol. *13*, 3–5, author reply 5–7.

Kuo, J.C., Han, X., Hsiao, C.T., Yates, J.R., 3rd, and Waterman, C.M. (2011). Analysis of the myosin-II-responsive focal adhesion proteome reveals a role for β-Pix in negative regulation of focal adhesion maturation. Nat. Cell Biol. *13*, 383–393.

Lewis, W.H. (1922). The adhesive quality of cells. Anat. Rec. *23*, 387–392.

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

Ma, Y.Q., Qin, J., Wu, C., and Plow, E.F. (2008). Kindlin-2 (Mig-2): a co-activator of beta3 integrins. J. Cell Biol. *181*, 439–446.

Machacek, M., Hodgson, L., Welch, C., Elliott, H., Pertz, O., Nalbant, P., Abell, A., Johnson, G.L., Hahn, K.M., and Danuser, G. (2009). Coordination of Rho GTPase activities during cell protrusion. Nature *461*, 99–103.

Margadant, F., Chew, L.L., Hu, X., Yu, H., Bate, N., Zhang, X., and Sheetz, M. (2011). Mechanotransduction in vivo by repeated talin stretch-relaxation events depends upon vinculin. PLoS Biol. *9*, e1001223.

McCann, R.O., and Craig, S.W. (1999). Functional genomic analysis reveals the utility of the I/LWEQ module as a predictor of protein:actin interaction. Biochem. Biophys. Res. Commun. *266*, 135–140.

Medalia, O., and Geiger, B. (2010). Frontiers of microscopy-based research into cell-matrix adhesions. Curr. Opin. Cell Biol. *22*, 659–668.

Montanez, E., Ussar, S., Schifferer, M., Bösl, M., Zent, R., Moser, M., and Fässler, R. (2008). Kindlin-2 controls bidirectional signaling of integrins. Genes Dev. *22*, 1325–1330.

Moore, S.W., Roca-Cusachs, P., and Sheetz, M.P. (2010). Stretchy proteins on stretchy substrates: the important elements of integrin-mediated rigidity sensing. Dev. Cell *19*, 194–206.

Moser, M., Nieswandt, B., Ussar, S., Pozgajova, M., and Fässler, R. (2008). Kindlin-3 is essential for integrin activation and platelet aggregation. Nat. Med. *14*, 325–330.

Na, S., Collin, O., Chowdhury, F., Tay, B., Ouyang, M., Wang, Y., and Wang, N. (2008). Rapid signal transduction in living cells is a unique feature of mechanotransduction. Proc. Natl. Acad. Sci. USA *105*, 6626–6631.

Nandrot, E.F., Kim, Y., Brodie, S.E., Huang, X., Sheppard, D., and Finnemann, S.C. (2004). Loss of synchronized retinal phagocytosis and age-related blindness in mice lacking alphavbeta5 integrin. J. Exp. Med. *200*, 1539–1545.

Nimnual, A.S., Taylor, L.J., and Bar-Sagi, D. (2003). Redox-dependent downregulation of Rho by Rac. Nat. Cell Biol. *5*, 236–241.

Nobes, C.D., and Hall, A. (1995). Rho, rac, and cdc42 GTPases regulate the assembly of multimolecular focal complexes associated with actin stress fibers, lamellipodia, and filopodia. Cell *81*, 53–62.

Oakes, P.W., Beckham, Y., Stricker, J., and Gardel, M.L. (2012). Tension is required but not sufficient for focal adhesion maturation without a stress fiber template. J. Cell Biol. *196*, 363–374.

Pankov, R., Cukierman, E., Katz, B.Z., Matsumoto, K., Lin, D.C., Lin, S., Hahn, C., and Yamada, K.M. (2000). Integrin dynamics and matrix assembly: tensin-dependent translocation of alpha(5)beta(1) integrins promotes early fibronectin fibrillogenesis. J. Cell Biol. *148*, 1075–1090.

Pasapera, A.M., Schneider, I.C., Rericha, E., Schlaepfer, D.D., and Waterman, C.M. (2010). Myosin II activity regulates vinculin recruitment to focal adhesions through FAK-mediated paxillin phosphorylation. J. Cell Biol. *188*, 877–890.

Patla, I., Volberg, T., Elad, N., Hirschfeld-Warneken, V., Grashoff, C., Fässler, R., Spatz, J.P., Geiger, B., and Medalia, O. (2010). Dissecting the molecular architecture of integrin adhesion sites by cryo-electron tomography. Nat. Cell Biol. *12*, 909–915.

Pelham, R.J., Jr., and Wang, Yl. (1997). Cell locomotion and focal adhesions are regulated by substrate flexibility. Proc. Natl. Acad. Sci. USA *94*, 13661–13665.

Pines, M., Das, R., Ellis, S.J., Morin, A., Czerniecki, S., Yuan, L., Klose, M., Coombs, D., and Tanentzapf, G. (2012). Mechanical force regulates integrin turnover in Drosophila in vivo. Nat. Cell Biol. *14*, 935–943.

Ponti, A., Machacek, M., Gupton, S.L., Waterman-Storer, C.M., and Danuser, G. (2004). Two distinct actin networks drive the protrusion of migrating cells. Science *305*, 1782–1786.

Prager-Khoutorsky, M., Lichtenstein, A., Krishnan, R., Rajendran, K., Mayo, A., Kam, Z., Geiger, B., and Bershadsky, A.D. (2011). Fibroblast polarization is a matrix-rigidity-dependent process controlled by focal adhesion mechanosensing. Nat. Cell Biol. *13*, 1457–1465.

Rappaport, R., and Rappaport, B.N. (1968). An analysis of cytokinesis in cultured newt cells. J. Exp. Zool. *168*, 187–195.

Rees, D.A., Lloyd, C.W., and Thom, D. (1977). Control of grip and stick in cell adhesion through lateral relationships of membrane glycoproteins. Nature *267*, 124–128.

Revel, J.P., and Wolken, K. (1973). Electronmicroscope investigations of the underside of cells in culture. Exp. Cell Res. *78*, 1–14.

Riveline, D., Zamir, E., Balaban, N.Q., Schwarz, U.S., Ishizaki, T., Narumiya, S., Kam, Z., Geiger, B., and Bershadsky, A.D. (2001). Focal contacts as mechanosensors: externally applied local mechanical force induces growth of focal contacts by an mDia1-dependent and ROCK-independent mechanism. J. Cell Biol. *153*, 1175–1186.

Roca-Cusachs, P., Gauthier, N.C., Del Rio, A., and Sheetz, M.P. (2009). Clustering of alpha(5)beta(1) integrins determines adhesion strength whereas alpha(v)beta(3) and talin enable mechanotransduction. Proc. Natl. Acad. Sci. USA *106*, 16245–16250.

Rogalski, T.M., Mullen, G.P., Gilbert, M.M., Williams, B.D., and Moerman, D.G. (2000). The UNC-112 gene in Caenorhabditis elegans encodes a novel component of cell-matrix adhesion structures required for integrin localization in the muscle cell membrane. J. Cell Biol. *150*, 253–264.

Rohrschneider, L.R. (1980). Adhesion plaques of Rous sarcoma virus-transformed cells contain the src gene product. Proc. Natl. Acad. Sci. USA *77*, 3514–3518.

Rottner, K., Hall, A., and Small, J.V. (1999). Interplay between Rac and Rho in the control of substrate contact dynamics. Curr. Biol. *9*, 640–648.

Rous, P. (1911). A sarcoma of the fowl transmissible by an agent separable from the tumor cells. J. Exp. Med. *13*, 397–411.

Sakai, R., Iwamatsu, A., Hirano, N., Ogawa, S., Tanaka, T., Mano, H., Yazaki, Y., and Hirai, H. (1994). A novel signaling molecule, p130, forms stable complexes in vivo with v-Crk and v-Src in a tyrosine phosphorylation-dependent manner. EMBO J. *13*, 3748–3756.

Samuel, M.S., Lopez, J.I., McGhee, E.J., Croft, D.R., Strachan, D., Timpson, P., Munro, J., Schröder, E., Zhou, J., Brunton, V.G., et al. (2011). Actomyosin-mediated cellular tension drives increased tissue stiffness and β-catenin activation to induce epidermal hyperplasia and tumor growth. Cancer Cell *19*, 776–791.

Sanes, J.R. (1983). Roles of extracellular matrix in neural development. Annu. Rev. Physiol. *45*, 581–600.

Sanford, K.K., Dunn, T.B., Covalesky, A.B., Dupree, L.T., and Earle, W.R. (1961). Polyoma virus and production of malignancy in vitro. J. Natl. Cancer Inst. *26*, 331–357.

Sawada, Y., Tamada, M., Dubin-Thaler, B.J., Cherniavskaya, O., Sakai, R., Tanaka, S., and Sheetz, M.P. (2006). Force sensing by mechanical extension of the Src family kinase substrate p130Cas. Cell *127*, 1015–1026.

Schiller, H.B., Friedel, C.C., Boulegue, C., and Fässler, R. (2011). Quantitative proteomics of the integrin adhesome show a myosin II-dependent recruitment of LIM domain proteins. EMBO Rep. *12*, 259–266.

Schlunck, G., Han, H., Wecker, T., Kampik, D., Meyer-ter-Vehn, T., and Grehn, F. (2008). Substrate rigidity modulates cell matrix interactions and protein expression in human trabecular meshwork cells. Invest. Ophthalmol. Vis. Sci. *49*, 262–269.

Schmits, R., Kündig, T.M., Baker, D.M., Shumaker, G., Simard, J.J., Duncan, G., Wakeham, A., Shahinian, A., van der Heiden, A., Bachmann, M.F., et al. (1996). LFA-1-deficient mice show normal CTL responses to virus but fail to reject immunogenic tumor. J. Exp. Med. *183*, 1415–1426.

Schvartzman, M., Palma, M., Sable, J., Abramson, J., Hu, X., Sheetz, M.P., and Wind, S.J. (2011). Nanolithographic control of the spatial organization of cellular adhesion receptors at the single-molecule level. Nano Lett. *11*, 1306–1312.

Sequeira, S.J., Soscia, D.A., Oztan, B., Mosier, A.P., Jean-Gilles, R., Gadre, A., Cady, N.C., Yener, B., Castracane, J., and Larsen, M. (2012). The regulation of focal adhesion complex formation and salivary gland epithelial cell organization by nanofibrous PLGA scaffolds. Biomaterials *33*, 3175–3186.

Shemesh, T., Geiger, B., Bershadsky, A.D., and Kozlov, M.M. (2005). Focal adhesions as mechanosensors: a physical mechanism. Proc. Natl. Acad. Sci. USA *102*, 12383–12388.

Siegel, D.H., Ashton, G.H., Penagos, H.G., Lee, J.V., Feiler, H.S., Wilhelmsen, K.C., South, A.P., Smith, F.J., Prescott, A.R., Wessagowit, V., et al. (2003). Loss of kindlin-1, a human homolog of the Caenorhabditis elegans actin-extracellular-matrix linker protein UNC-112, causes Kindler syndrome. Am. J. Hum. Genet. *73*, 174–187.

Singer, I.I. (1979). The fibronexus: a transmembrane association of fibronectin-containing fibers and bundles of 5 nm microfilaments in hamster and human fibroblasts. Cell *16*, 675–685.

Stoker, M., O'Neill, C., Berryman, S., and Waxman, V. (1968). Anchorage and growth regulation in normal and virus-transformed cells. Int. J. Cancer *3*, 683–693.

Temin, H.M., and Rubin, H. (1958). Characteristics of an assay for Rous sarcoma virus and Rous sarcoma cells in tissue culture. Virology *6*, 669–688.

Théry, M., Racine, V., Piel, M., Pépin, A., Dimitrov, A., Chen, Y., Sibarita, J.B., and Bornens, M. (2006). Anisotropy of cell adhesive microenvironment governs cell internal organization and orientation of polarity. Proc. Natl. Acad. Sci. USA *103*, 19771–19776.

Thiery, J.P., Duband, J.L., and Tucker, G.C. (1985). Cell migration in the vertebrate embryo: role of cell adhesion and tissue environment in pattern formation. Annu. Rev. Cell Biol. *1*, 91–113.

Thom, D., Powell, A.J., and Rees, D.A. (1979). Mechanisms of cellular adhesion. IV. Role of serum glycoproteins in fibroblast spreading on glass. J. Cell Sci. *35*, 281–305.

Trappmann, B., Gautrot, J.E., Connelly, J.T., Strange, D.G., Li, Y., Oyen, M.L., Cohen Stuart, M.A., Boehm, H., Li, B., Vogel, V., et al. (2012). Extracellular-matrix tethering regulates stem-cell fate. Nat. Mater. *11*, 642–649.

Ussar, S., Moser, M., Widmaier, M., Rognoni, E., Harrer, C., Genzel-Boroviczeny, O., and Fässler, R. (2008). Loss of Kindlin-1 causes skin atrophy and lethal neonatal intestinal epithelial dysfunction. PLoS Genet. *4*, e1000289.

Vakaloglou, K.M., Chountala, M., and Zervas, C.G. (2012). Functional analysis of parvin and different modes of IPP-complex assembly at integrin sites during Drosophila development. J. Cell Sci. *125*, 3221–3232.

Vicente-Manzanares, M., and Horwitz, A.R. (2011). Adhesion dynamics at a glance. J. Cell Sci. *124*, 3923–3927.

Vogel, V., and Sheetz, M.P. (2009). Cell fate regulation by coupling mechanical cycles to biochemical signaling pathways. Curr. Opin. Cell Biol. *21*, 38–46.

Volk, T., Fessler, L.I., and Fessler, J.H. (1990). A role for integrin in the formation of sarcomeric cytoarchitecture. Cell *63*, 525–536.

Weinstein, A. (1918). Coincidence of Crossing over in DROSOPHILA MELANOGASTER (AMPELOPHILA). Genetics *3*, 135–172.

Wilcox, M. (1990). Genetic analysis of the Drosophila PS integrins. Cell Differ. Dev. *32*, 391–399.

Wolfenson, H., Henis, Y.I., Geiger, B., and Bershadsky, A.D. (2009a). The heel and toe of the cell's foot: a multifaceted approach for understanding the structure and dynamics of focal adhesions. Cell Motil. Cytoskeleton *66*, 1017–1029.

Wolfenson, H., Lubelski, A., Regev, T., Klafter, J., Henis, Y.I., and Geiger, B. (2009b). A role for the juxtamembrane cytoplasm in the molecular dynamics of focal adhesions. PLoS ONE *4*, e4304.

Wolfenson, H., Bershadsky, A., Henis, Y.I., and Geiger, B. (2011). Actomyosin-generated tension controls the molecular kinetics of focal adhesions. J. Cell Sci. *124*, 1425–1432.

Worthington, J.J., Klementowicz, J.E., and Travis, M.A. (2011). TGFβ: a sleeping giant awoken by integrins. Trends Biochem. Sci. *36*, 47–54.

Yamaguchi, Y., Katoh, H., Yasui, H., Mori, K., and Negishi, M. (2001). RhoA inhibits the nerve growth factor-induced Rac1 activation through Rho-associated kinase-dependent pathway. J. Biol. Chem. *276*, 18977–18983.

Yarwood, S.J., and Woodgett, J.R. (2001). Extracellular matrix composition determines the transcriptional response to epidermal growth factor receptor activation. Proc. Natl. Acad. Sci. USA *98*, 4472–4477.

Yu, C.H., Law, J.B., Suryana, M., Low, H.Y., and Sheetz, M.P. (2011). Early integrin binding to Arg-Gly-Asp peptide activates actin polymerization and contractile movement that stimulates outward translocation. Proc. Natl. Acad. Sci. USA *108*, 20585–20590.

Zaidel-Bar, R., and Geiger, B. (2010). The switchable integrin adhesome. J. Cell Sci. *123*, 1385–1388.

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.

Zaidel-Bar, R., Cohen, M., Addadi, L., and Geiger, B. (2004). Hierarchical assembly of cell-matrix adhesion complexes. Biochem. Soc. Trans. *32*, 416–420.

Zaidel-Bar, R., Itzkovitz, S., Ma'ayan, A., Iyengar, R., and Geiger, B. (2007a). Functional atlas of the integrin adhesome. Nat. Cell Biol. *9*, 858–867.

Zaidel-Bar, R., Milo, R., Kam, Z., and Geiger, B. (2007b). A paxillin tyrosine phosphorylation switch regulates the assembly and form of cell-matrix adhesions. J. Cell Sci. *120*, 137–148.

Zamir, E., Katz, M., Posen, Y., Erez, N., Yamada, K.M., Katz, B.Z., Lin, S., Lin, D.C., Bershadsky, A., Kam, Z., and Geiger, B. (2000). Dynamics and segregation of cell-matrix adhesions in cultured fibroblasts. Nat. Cell Biol. *2*, 191–196.

Zervas, C.G., Gregory, S.L., and Brown, N.H. (2001). Drosophila integrin-linked kinase is required at sites of integrin adhesion to link the cytoskeleton to the plasma membrane. J. Cell Biol. *152*, 1007–1018.

Zervas, C.G., Psarra, E., Williams, V., Solomon, E., Vakaloglou, K.M., and Brown, N.H. (2011). A central multifunctional role of integrin-linked kinase at muscle attachment sites. J. Cell Sci. *124*, 1316–1327.