Abstract  Integrins are a large family of heterodimeric glycoprotein receptors first
discovered over twenty years ago. They exist as two noncovalently bound α- and
β- subunits that function as adhesion molecules and play key roles in many biological
processes including actin cytoskeleton organization and transduction of intracellular
signals regulating cellular functions. Integrins bind a variety of extracellular matrices
including collagens and laminins. The phenotypes observed from the generation of
integrin knockout mice have provided a wealth of information on the unique biological
functions of specific integrin heterodimers. Structural data obtained from X-ray crys-
tallography and nuclear magnetic resonance (NMR) have provided insight into the
structural basis for integrin activation and subsequent transduction of bidirectional
signals bidirectionally, important for controlling biological cellular functions.

Keywords  Cell-Extracellular matrix interactions • Cell signaling • Cell adhesion
• Cell migration

General Introduction

Integrins are a large family of type I transmembrane heterodimeric glycoprotein
receptors that function as the major metazoan receptors for cell adhesion and
connect the intracellular and extracellular environments. Integrins are found in
organisms ranging from sponges, corals, nematodes, and echinoderms to mammals
(Burke 1999). Integrins exist as two noncovalently bound α and β subunits, which
pair to form heterodimers. There are 18α and 8β known subunits which combine to
form at least 24 distinct integrin heterodimers (Hynes 2002). Each heterodimer
consists of a large extracellular domain which binds proteins in the extracellular
environment, a single-membrane-spanning transmembrane domain, and a generally

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short intracellular cytoplasmic tail domain, which forms links with the cytoskeletal elements via cytoplasmic adaptor proteins (Hynes 2002). Integrins can bind to extracellular matrix (ECM) glycoproteins including collagens, fibronectins, laminins, and cellular receptors such as vascular cell adhesion molecule-1 (VCAM-1) and the intercellular cell adhesion molecule (ICAM) family (Hynes 2002; Plow et al. 2000). In addition, integrins also play key roles in the assembly of the actin cytoskeleton as well as in modulating signal transduction pathways that control biological and cellular functions including cell adhesion, migration, proliferation, cell differentiation, and apoptosis (Schwartz et al. 1995). Since their discovery approximately 20 years ago, significant progress has been made in the integrin biology field that has resulted in a greatly improved understanding of their structure and function. In this chapter, we discuss the in vivo work with integrin knockout mice that has led to an improved understanding of the biological significance of integrins. We also discuss how the unique structure of integrins enables these cell receptors to signal bidirectionally, a critical function of integrins. Finally we will describe the fundamental mechanisms by which integrins assemble and link to the actin cytoskeleton as well how they transduce intracellular signals to modulate cellular functions.

**Classification of Integrins**

The specificity of integrin binding to ECM components including laminins, collagens, and fibronectin depends on the extracellular domains of the α and β integrin subunits (Fig. 2.1). Integrins α1β1, α2β1, α10β1, and α11β1 represent the primary collagen receptors (Hynes 2002; Ruggiero et al. 1996; Camper et al. 1998; Velling et al. 1999); integrins α3β1, α6β1, α6β4 and α7β1 are the major laminin receptors (Tashiro et al. 1999); and integrins α5β1, α8β1, αIIbβ3 and the αvβ integrins are the major fibronectin receptors that bind in an RGD-dependent manner (van der Flier and Sonnenberg 2001). Redundancy exists with respect to certain integrin–ECM interactions as some integrins bind the same extracellular ligands albeit at different affinities and conversely, some ligands are recognized by different integrins. For example integrins α1β1, α2β1, α10β1, and α11β1 all bind collagen, yet integrin α1β1 also binds laminins. Most integrin heterodimers are widely expressed in many tissues; however, some are more restricted in their expression. For example, αIIbβ3 is only found on platelets, α6β4 on keratinocytes, and αEβ7, α4β7, α4β1, and the β2 integrin families are restricted to leukocytes (Takada et al. 2007).

**Phenotypes of Integrin Knockout Mice**

Integrins play diverse and important roles in most biological processes (Hynes 2002). Mice that lack integrin expression either constitutively or in specific cell types exhibit a wide range of phenotypes. These knockout mice have provided...
much insight into the functions of specific integrin heterodimers, reflecting the unique roles of the various integrins. In general, each of the 24 integrins has a specific, nonredundant function. Genes for each of the β subunits and all but two of the α subunits have been deleted in mice (Table 2.1). Integrin β1 is ubiquitously expressed and can bind multiple α partners, and thus it is not surprising that knockout of β1 results in embryonic lethality due to a complete block in preimplantation development. In contrast, knockouts of α1, α2, α10, and α11 integrin subunits, which each exclusively heterodimerize with β1 to function as primary collagen receptors, are all viable and fertile but possess distinct characteristic abnormalities. For example, α1 integrin knockout mice develop increased collagen synthesis and display reduced tumor vascularization (Gardner et al. 1999; Pozzi and Zent 2003), while the α2 integrin knockout mouse has a more subtle phenotype with mild platelet function abnormalities and increased vascularization following wounding (Chen et al. 2002). These findings support the idea that there is significant redundancy and compensation amongst the collagen receptor integrins. In contrast when the primary laminin-binding (α3β1, α6β1/α6β4, α4β7) and the primary RGD-binding integrins (α5β1, α8β1, αV and αIIbβ3) are deleted, the phenotypes are more severe, suggesting less redundancy and compensation.
Mutations in integrin subunits have been found to cause clinical disorders in man and these correlate well with mice in which the same integrins are deleted. There are three well-described inherited autosomal recessive diseases in humans linked to germline mutations in integrin subunits. Mutations in \( \alpha_{IIb} \) and \( \beta_3 \) integrin subunits are associated with Glanzmann’s thrombasthenia, a clinical entity associated with platelet dysfunction and bleeding disorders (Hogg and Bates 2000). Both the \( \beta_3 \) and \( \alpha_{IIb} \) knockout mice exhibit features of defective platelet aggregation similar to that observed in Glanzmann’s thrombasthenia (Hogg and Bates 2000; Tronik-Le Roux et al. 2000; Hodivala-Dilke et al. 1999). Point mutations and gene deletion in \( \beta_2 \) integrin in humans and mice, respectively, have been associated with Leukocyte Adhesion Deficiency (LAD) (Hogg and Bates 2000; Scharffetter-Kochanek et al. 1998). Finally mutations in \( \alpha_6 \) and \( \beta_4 \) integrin result in junctional epidermolysis bullosa with skin blistering (Pulkkinen et al., 1997, 1998; Ruzzi et al. 1997; Takizawa et al. 1997; Vidal et al. 1995) and a similar phenotype is seen in mice lacking these integrins (Georges-Labouesse et al. 1996; van der Neut et al. 1996).

### Table 2.1 Classification of the phenotypes in integrin knockout mice

<table>
<thead>
<tr>
<th>Gene</th>
<th>Phenotype</th>
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<tbody>
<tr>
<td>( \alpha_1 )</td>
<td>( \text{V,F} ) Increased collagen synthesis, reduced tumor vascularization</td>
</tr>
<tr>
<td>( \alpha_2 )</td>
<td>( \text{V,F} ) Few developmental defects. Delayed platelet aggregation</td>
</tr>
<tr>
<td>( \alpha_3 )</td>
<td>( \text{L,birth} ) Defects in kidneys, lungs, and cerebral cortex; skin blistering</td>
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<tr>
<td>( \alpha_4 )</td>
<td>( \text{L,E11–E14} ) Defects in chorioallantois fusion and cardiac development</td>
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<tr>
<td>( \alpha_5 )</td>
<td>( \text{L,E10} ) Defects in extraembryonic and embryonic vascular development</td>
</tr>
<tr>
<td>( \alpha_6 )</td>
<td>( \text{L,birth} ) Defects in cerebral cortex and retina; skin blistering</td>
</tr>
<tr>
<td>( \alpha_7 )</td>
<td>( \text{V,F} ) Muscular dystrophy</td>
</tr>
<tr>
<td>( \alpha_8 )</td>
<td>( \text{L+V/F} ) Small or absent kidneys; inner ear defects</td>
</tr>
<tr>
<td>( \alpha_9 )</td>
<td>( \text{L, perinatal} ) Bilateral chylothorax</td>
</tr>
<tr>
<td>( \alpha_{10} )</td>
<td>( \text{V,F} ) Dysfunction of growth plate chondrocytes</td>
</tr>
<tr>
<td>( \alpha_{11} )</td>
<td>( \text{V,F} ) Dwarfism most likely resulting from severely defective incisors</td>
</tr>
<tr>
<td>( \alpha_{12} )</td>
<td>( \text{L,E12-birth} ) Defects in placenta and in CNS and GI blood vessels; cleft palate</td>
</tr>
<tr>
<td>( \alpha_{13} )</td>
<td>( \text{…} ) No reported knockout available</td>
</tr>
<tr>
<td>( \alpha_{14} )</td>
<td>( \text{V,F} ) Impaired leukocyte recruitment and tumor rejection</td>
</tr>
<tr>
<td>( \alpha_{M} )</td>
<td>( \text{V,F} ) Impaired phagocytosis and PMN apoptosis; obesity; mast cell development</td>
</tr>
<tr>
<td>( \alpha_{x} )</td>
<td>( \text{…} ) No reported knockout available</td>
</tr>
<tr>
<td>( \alpha_{y} )</td>
<td>( \text{V,F} ) Inflammatory skin lesions</td>
</tr>
<tr>
<td>( \alpha_{m} )</td>
<td>( \text{V,F} ) Defective platelet aggregation</td>
</tr>
<tr>
<td>( \beta_1 )</td>
<td>( \text{L,E5.5} ) Inner cell mass deterioration</td>
</tr>
<tr>
<td>( \beta_2 )</td>
<td>( \text{V,F} ) Impaired leukocyte recruitment; skin infections</td>
</tr>
<tr>
<td>( \beta_3 )</td>
<td>( \text{V,F} ) Defective platelet aggregation; osteosclerosis</td>
</tr>
<tr>
<td>( \beta_4 )</td>
<td>( \text{L,perinatal} ) Skin blistering</td>
</tr>
<tr>
<td>( \beta_5 )</td>
<td>( \text{V,F} ) No apparent phenotype</td>
</tr>
<tr>
<td>( \beta_6 )</td>
<td>( \text{V,F} ) Skin and lung inflammation and impaired lung fibrosis</td>
</tr>
<tr>
<td>( \beta_7 )</td>
<td>( \text{V,F} ) Abnormal Peyer’s patches; decreased no. of intraepithelial lymphocytes</td>
</tr>
<tr>
<td>( \beta_8 )</td>
<td>( \text{L,E12-birth} ) Defects in placenta and in CNS and GI blood vessels; cleft palate</td>
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Table adapted from Bouvard/Fassler et al. (2001 Circ Res)

V, viable; F, fertile; L, lethal; L+V/F, disrupted development in some but survival in a fraction of others
Structure

Each subunit of the integrin $\alpha\beta$ heterodimers contains a large extracellular domain, a single-spanning transmembrane domain, and a short cytoplasmic tail (with the exception of $\beta 4$). Over the past decade, we have begun to recognize and understand better the importance of integrins in mediating adhesion and signaling. This recognition has yielded the emergence of important structural information of integrin conformations. Solving high-resolution structures of integrins has proved challenging, particularly because integrins are large membrane proteins, and thus difficult to purify. Many years of effort have been devoted to understanding and characterizing integrin structure, and knowledge of the structural basis for integrins has helped gain insight to further characterize the mechanisms underlying integrin activation.

Extracellular Domain

The extracellular domain of integrins are generally large, ~80–150 kDa structures. Most of the structural data of the extracellular domains comes from high-resolution X-ray crystallography. The first extracellular domain crystal structure solved was the inserted (I)-domain from $\alpha\text{M}\beta 2$ in 1995 (Lee et al. 1995). It took six additional years to solve the first complete extracellular domain of an integrin ($\alpha\nu\beta 3$) (Xiong et al. 2001). The extracellular portion of the $\alpha$ and $\beta$ subunits are comprised of several subdomains organized into a globular ligand-binding N-terminal head domain standing on two long and extended C-terminal legs that connect to the transmembrane and cytoplasmic domains of each respective subunit (Fig. 2.2) (Nermut et al. 1988).

The $\alpha$ subunit head consists of a folded seven-bladed $\beta$ propeller head domain, a thigh domain and two calf domains (Xiong et al. 2001; Springer 1997). Half of the $\alpha$ subunits contain an additional inserted (I)-domain of ~200 amino acids that is inserted within the $\beta$ propeller domain. When present, the $\alpha$ I-domain represents the exclusive extracellular-binding site for ligands. The $\alpha$ I-domain contains a conserved “metal-ion-dependent adhesive site” (MIDAS) that binds divalent metal cations ($\text{Mg}^{2+}$) and plays important roles in protein ligand binding. Ligand binding alters the coordination of the metal ion and shifts the I-domain from a closed, resting state to an open, active conformation which results in increased ligand affinity and promotes subsequent integrin activation (Liddington and Ginsberg 2002). This mode of activation is analogous to small G proteins, whereby GTP hydrolysis leads to altered coordination of a $\text{Mg}^{2+}$ ion and subsequent conformation changes.

The $\beta$ subunit is composed of an I-like domain, which is structurally similar to the I-domain in $\alpha$ subunits, a PSI (plexin/semaphorin/integrin) domain, a hybrid domain, four EGF repeats, and a membrane proximal $\beta$ tail ($\beta$TD) domain. The $\beta$ subunit plays important roles in ligand binding in $\alpha$ subunits which lack the I-domain. In these integrin heterodimers, ligands bind to a crevice in the head domain between the $\alpha\beta$ subunit interfaces. The ligand interacts with a metal-ion-occupied MIDAS located within the $\beta$ subunit and the propeller domain of the $\alpha$ subunit.
Transmembrane Domain

The transmembrane (TM) domains of integrins are single spanning structures comprised of ~25–29 amino acid residues that form α-helical coiled coils that either homo- or heterodimerize (Adair and Yeager 2002). Unlike integrin extracellular domains, no high-resolution experimental X-ray crystal structures are available for the TM domain of any integrin heterodimer, and much of the structural data are based on NMR analysis. Structural information from the αIIbβ3 heterodimer TM domains have only recently been solved in their entirety (Adair and Yeager 2002; Lau et al., 2008a, 2008b, 2009). The αIIb TM domain is a 24 residue α-helix followed by a backbone reversal that lacks a significant helix tilt (Lau et al. 2008a). The distal GFF motif (depicted in bold in Figure 2.3) is highly conserved in the 18 human integrin α subunits and likely plays an important role in the transition from the resting to active states. The β3 TM domain is a 30-residue linear α helix that is somewhat longer than the width of a typical lipid bilayer, implying that a pronounced helix tilt is present within the plasma membrane (Lau et al. 2008b).
Inactive integrin TM subunits are tightly packed and form a complex consisting of a coiled-coil interaction between canonical GxxxG dimerization motifs within the TM domains (depicted in gold in Fig. 2.3) (Gottschalk 2005).

**Cytoplasmic Domain**

Integrin cytoplasmic domains are generally short, largely unstructured and comprised of 10–70 amino acid residues, with the exception of the β4 subunit which contains >1,000 amino acid residues. Like TM domains, no high-resolution X-ray crystal structures of the cytoplasmic domains have been solved, and much of the structural information is also based on NMR data. Only the structures of the αIIbβ3 cytoplasmic tails have been characterized under heterodimeric conditions (Li et al. 2001; Ulmer et al. 2001; Vinogradova et al. 2002; Weljie et al. 2002; Vinogradova et al. 2004). β cytoplasmic tails are highly homologous, while α subunit tails are highly divergent. The conserved GFFKR and HDR(K/E) sequences located in the membrane proximal regions of the α and β subunits, respectively, are proposed to form a salt bridge between arginine (R) from the α subunit and aspartic acid (D) from the β subunit (Adair and Yeager 2002; Vinogradova et al. 2000). In αIIbβ3 integrins, the salt bridge represents a physical interaction between αIIβ and β3 cytoplasmic tails (depicted in green in Fig. 2.3). Although not confirmed by structural data from other integrin heterodimers, the salt bridge is thought to be present in other integrin pairs and generally believed to function to maintain integrins in the inactive, low-affinity state. Disruption of the salt bridge in β3 integrins has been shown to play key roles in regulating integrin activation states (Hughes et al., 1995, 1996), however, in β1 integrins, disruption of the salt bridge via a point mutation in the aspartic acid residue of β1 did not result in a major phenotype in mice. These findings suggest that the salt bridge might not play a major role in β1 integrin activation (Czuchra et al. 2006).
Within the β integrin tails are two well-defined motifs: a membrane proximal NpxY and a membrane distal NxxY motif (depicted in red in Fig. 2.3). These motifs represent canonical recognition sequences for phosphotyrosine-binding (PTB) domains (Calderwood et al. 2003) and serve as binding sites for multiple integrin binding proteins, including talin and the kindlins.

**Integrin Signaling**

Integrins are able to transduce signals intracellularly following ligand binding (“outside-in” signaling). However, unlike most other cell receptors, integrins can shift between high- and low-affinity conformations for ligand binding (“inside-out” signaling). Depending on the cell type, integrins can be either basally activated, as with most adherent cells that are attached to a basement membrane, or basally inactive, as with platelets or leukocytes that freely circulate until activated to undergo platelet aggregation or mediate an inflammatory response, respectively. The αIIbβ3 integrin heterodimers located on the cell surface of platelets represent the best-characterized basally inactive integrins. In these cells, integrins exist in a low-affinity state with respect to extracellular ligand binding and rely on intracellular signaling to become activated.

Integrins themselves have no kinase activity but instead provide a connection between the extracellular matrix and the actin cytoskeleton. This connection allows integrins to regulate cytoskeletal organization and cell motility as well as to alter fluxes of many intracellular-signaling pathways including cell survival, cell proliferation, cell shape, and angiogenesis. The extracellular domains can bind a variety of ligands, whereas the intracellular cytoplasmic domains anchor cytoskeletal proteins. This linkage between the cell exterior and interior allows for bidirectional signaling across the plasma membrane.

**Inside-Out Signaling**

Inside-out signaling or integrin activation is important in physiological situations such as in the blood, where cells are in close proximity to their ligands, yet cell–ligand interactions occur only following integrin activation in response to specific external cues such as injury to the vasculature or the induction of inflammation (Ratnikov et al. 2005). This characteristic of integrin regulation is also important in the developmental processes when cells are required to migrate for specific periods of time during morphogenic processes.

In the normal resting, inactive state, integrin extracellular domains are unbound to ligands and exist in a bent conformation. Activation signals from within the cell induce straightening of the extracellular domains and stabilize the extended, active conformation. This conformational change exposes the external ligand-binding site to
Integrin Structure and Function

which ligands bind, allowing the transmission of signals from the outside to the inside (Fig. 2.2). The exact changes that occur in the extracellular head domain once integrins undergo conformational change to the high affinity state are still unclear. Two models of conformational change have been proposed. In the “switchblade” model (Luo et al. 2007), only extended integrins are predicted to bind ligand. In contrast is the “deadbolt model” (Xiong et al. 2003), which suggests that integrin extension occurs only after ligand binding has occurred. In both models conformational changes within the head domain facilitate ligand binding (Luo et al. 2007; Arnaout et al. 2007).

The TM domains play key roles in integrin activation. Separation of integrin TM domains is generally believed to be a requirement for integrins to adopt the high affinity state. Several possible models exist for disruption of TM domains (Wegener and Campbell 2008). In the piston model, vertical movements of the TM domains cause a shift in the domains, while a scissors model suggests there is an increase in the angle of the TM domains. Finally there is a separation model that predicts a physical separation of the two TM subunits. Regardless of the model, each result in disruption of the interaction between the two subunits, facilitating conformational changes in both the extracellular or cytoplasmic domains and subsequent integrin activation (Wegener and Campbell 2008). Recent data have shown that the dissociation of the α and β subunits in the TM complex is central to the extracellular conformational changes associated with integrin signaling (Lau et al. 2009).

The role of integrin cytoplasmic tails in the regulation of integrin affinity has been extensively examined in the rapidly activated αIIbβ3 and β2 integrin families (Wegener et al. 2007). A large number of cytoskeletal and signaling proteins bind to integrin cytoplasmic tails, however, only two proteins, talin and the kindlins, which both bind to β integrin cytoplasmic tails, have been demonstrated to be important for separation of the cytoplasmic tails and subsequent integrin activation. The membrane proximal NpxY motif is required for the binding of the cytoskeletal protein talin (Calderwood et al. 2003). The membrane distal NxxY motif is required for the binding of kindlins, which have recently been described to play a role in integrin activation (Montanez et al. 2008; Moser et al. 2008). The following sections discuss how talin and kindlins are thought to regulate integrin affinity.

Talin and Kindlin Binding are Required for Integrin Activation

Talin is an actin-binding protein that binds the proximal NpxY motif on β cytoplasmic tails and plays important roles in integrin activation and linkage to the actin cytoskeleton. Talins are large ~270 kDa proteins comprised of an N-terminal head domain (~47 kDa) and a large tail rod region (~220 kDa). The head domain is comprised of a FERM (4.1, ezrin, radixin, moesin) domain containing three subdomains (F1, F2, F3) which binds integrin cytoplasmic tails. The tail region contains multiple binding sites for vinculin as well as an additional binding site for integrin cytoplasmic tails. Talin is an essential mediator of integrin activation and has been shown to induce activation of the normally inactive platelet integrin αIIbβ3
Integrin activation by talin is thought to occur by competition with the αIIb tail for binding to the β3 tail on αIIbβ3 integrin. The F3 subdomain of talin resembles a phosphotyrosine binding (PTB) domain and binds the membrane proximal NpxY motif of β integrin tails, leading to destabilization of the putative integrin salt bridge. The net result is a change in the position of the TM helix, leading to a packing mismatch within the αIIbβ3-TM helix, and separation or reorientation of the integrin tails (Luo et al. 2004, 2005, 2007; Yin et al. 2006; Loh et al. 1996; Li et al. 2003, 2004, 2005; Partridge et al. 2005). These events result in conformational change of the extracellular domain, leading to increased ligand affinity and integrin activation. Although other PTB domain-containing proteins (Dok1, tensin) can also bind the proximal NpxY motif on β tails, only talin modulates integrin activation. This is thought to be because the talin F3 subdomain binds to an additional, more proximal site on β integrin tails, which induces displacement of α and β integrin tails (Wegener et al. 2007).

As integrin activation is strictly controlled, the binding of talin to integrins is tightly regulated. Under normal physiologic conditions, talin exists in an autoinhibited state, unable to bind integrins. The talin C-terminus interacts with the talin PTB domain, blocking the integrin-binding pocket. Additionally, talins also homodimerize (Goldmann et al. 1994) and the intermolecular interaction in turn also masks integrin-binding sites (Ratnikov et al. 2005). The mechanisms of talin activation are unclear but likely involve the phosphotidylinositol-4,5-bisphosphate (PIP2) lipid second messengers. Talin binds and activates PIPKIγ which results in increased production of PIP2. PIP2 regulates talin, vinculin and other focal adhesion (FA) proteins, which leads to integrin activation, reinforcement of the cytoskeleton, intracellular signaling, and FA formation. In addition talin–integrin interactions are also controlled through tyrosine phosphorylation of the NpxY motif of β integrin tails by src family kinases (Law et al. 1996; Sakai et al. 2001).

Kindlins have recently been shown to play an important role in integrin inside-out signaling. Kindlins are a novel family of evolutionarily conserved adaptor proteins named after the gene mutated in Kindler syndrome in humans, a rare disease caused by mutations in kindlin-1 and characterized by skin blistering. Kindlin-mediated integrin activation requires a direct interaction between kindlin and β integrin tails. Like talin, kindlins also contain a FERM domain that has high levels of sequence similarity to talin FERM domains (Kloeker et al. 2004) and also represents the site of binding to β integrin tails. Kindlins bind the distal NxxY motif on β1, β2 and β3 integrin tails (Montanez et al. 2008; Moser et al. 2008; Ma et al. 2008; Shi et al. 2007; Ussar et al. 2008; Moser et al. 2009) (Fig. 2.3) but additional sequences may also be involved in kindlin binding. Although the intervening sequence between the two NxxY motifs in the β1 and β3 integrin cytoplasmic tails are dispensable for talin binding, mutation of a double Thr or Ser/Thr within this sequence impairs kindlin binding (depicted in blue in Fig. 2.3) (Moser et al. 2008). Both kindlins and talin bind distinct regions of the β integrin tails and together cooperate to regulate integrin affinity (Montanez et al. 2008; Moser et al. 2008;
Calderwood et al., 1999, 2002; Ma et al. 2008; Moser et al. 2009). Kindlins facilitate talin function, and expression of kindlin alone is insufficient to shift integrins to a high affinity state. In vitro studies have demonstrated that the amount of talin expressed in cells determines the efficacy of kindlins in promoting this function (Ma et al. 2008). Conversely, talin depends on kindlins to promote integrin affinity (Montanez et al. 2008). Thus both kindlins and talin are required to increase integrin affinity and promote integrin activation.

**Outside-In Signaling**

Integrins themselves lack intrinsic catalytic activity. Ligand binding to the extracellular domain of integrins results in signal transduction to the cytoplasm in the classical direction from the outside-in. These intracellular signals affect cellular growth, differentiation and apoptosis. Integrin signaling is complex and significantly influenced by crosstalk with growth factor receptors. Further, the intracellular signals generated lead to the assembly of the FA complex, a large, dynamic multiprotein complex involving over 150 intracellular proteins (Zaidel-Bar et al. 2007). FAs serve as the hub for transmission of intracellular signals. Within FAs, proteins are in constant flux, continually associating and dissociating with each other. The pathways following activation of multiple signaling cascades are complex and a schematic detailing of the complexities involved is shown in Fig. 2.4.

**Integrins are Essential to FA Formation and Assembly of the Actin Cytoskeleton**

Extracellular ligand binding leads to integrin clustering in the plane of the plasma membrane and the generation of tight FAs which promotes assembly of actin filaments. Actin filaments are reorganized into larger stress fibers leading to further integrin clustering and enhanced matrix binding (Giancotti and Ruoslahti 1999). In this manner, integrin linkage to the actin cytoskeleton allows regulation of FA growth as well as regulation of cell shape via the spatiotemporal control of cell protrusion and retraction during cell migration. Several key FA proteins are involved in establishing and maintaining the integrin–cytoskeleton linkage. These include (1) integrin-bound proteins that can directly bind to actin (talin, α-actinin, filamin); (2) integrin-bound proteins that indirectly bind the cytoskeleton (kindlins, integrin-linked kinase (ILK), paxillin, FAK); (3) non-integrin-bound actin-binding proteins (vinculin); and (4) adaptor and signaling molecules that regulate the interactions of the proteins from the above-mentioned groups. It is beyond the scope of this chapter to review in detail the complex machinery involved in the assembly of the actin cytoskeleton and the reader is referred to several recent excellent reviews (Geiger et al. 2009; Legate et al. 2009).
The initial integrin–cytoskeleton linkage following integrin ligation involves the recruitment of talin to β integrins. In contrast to integrin activation, which requires only the FERM domain in the talin head region, integrin–cytoskeletal linkage requires both the head and rod domains of talins. Talin provides a crucial connection to the actin cytoskeleton as mice with deletions of talin-1 die during gastrulation due to a defect in cytoskeletal organization and cell migration (Monkley et al. 2000). Further evidence for the importance of talin in cytoskeletal organization comes from flies lacking talin. In these animals integrins are able to associate with the ECM but are unable to link to the cytoskeleton, thus resulting in muscle detachment (Brown et al. 2002).
Vinculin

Following talin binding, proteins such as vinculin are recruited to the nascent focal adhesion. Vinculin does not bind integrins directly; rather it binds to several sites on the talin rod and to actin and is thought to act as a crosslinker that stabilizes the talin–actin interaction (Gallant et al. 2005; Humphries et al. 2007). Vinculin knockout fibroblasts make fewer and smaller FAs which are unable to mature (Saunders et al. 2006), providing evidence that vinculin is required to reinforce the link between integrins and the actin cytoskeleton.

α-Actinin

α-Actinin, a binding partner of both talin and vinculin, is another critical protein that links integrins to the cytoskeleton. α-actinin can directly bind β integrins and operates as a close partner of actin and has been shown to have an essential role in adhesion strengthening (Brakebusch and Fassler 2003). Flies lacking α-actinin die due to defects in muscle structure and function (Fyrberg et al. 1998) and mutations of the 4 α actinin isoforms in mice result in abnormalities in different organs depending on the isoform deleted (Craig et al. 2007; Weins et al. 2007).

Integrin-Linked Kinase

Integrin-linked kinase (ILK) is another important scaffolding protein which links integrins to the actin cytoskeleton. ILK is a multidomain adaptor protein that directly binds β1 and β3 integrin tails and indirectly associates with actin through its main binding partner, parvin. ILK also binds to the cytoskeleton through its associations with paxillin, which also binds parvin and vinculin (Legate et al. 2006). Cells deficient in ILK demonstrate severe delays in focal adhesion formation resulting in defective cell spreading (Sakai et al. 2003). Deletion of ILK from the skeletal muscle results in detachment of basement membranes and accumulation of extracellular matrix, providing further evidence that ILK plays critical roles in stabilizing the integrin–actin interaction (Wang et al. 2008).

Focal Adhesion Kinase

Focal adhesion kinase (FAK) is a nonreceptor tyrosine kinase signaling protein that most likely interacts indirectly with β integrins through its association with paxillin (Geiger et al. 2009; Brakebusch and Fassler 2003). Although not required for nascent FA formation or for the initial connection of integrins to the actin cytoskeleton, FAK is required to stabilize the linkage to actin by modulating the affinity of α-actinin to actin (Ilic et al. 1995), which is thought to occur via FAK-mediated phosphorylation of α-actinin. In addition to cytoskeletal stabilization, FAK also plays an essential role in promoting FA turnover (Ilic et al. 1995).
Kindlins

Kindlins, which were discussed earlier, directly bind β1 and β3 integrins and connect them to the actin cytoskeleton via a migfilin–filamin interaction as well as through the ILK–parvin complex (Larjava et al. 2008). Mouse platelets lacking kindlin-3 are unable to organize their cytoskeleton or establish stable lamellipodia (Moser et al. 2008), providing evidence for the importance of the kindlin interactions with the cytoskeleton. In addition loss of kindlin-2 results in early embryonic lethality and impaired actin polarization (Montanez et al. 2008), and loss of kindlin-1 results in shear-induced detachment of intestinal epithelial cells (Ussar et al. 2008).

Paxillin and Tensin

Paxillin and tensin are two important integrin–actin regulatory proteins. Paxillin is detected early in nascent adhesions at the leading edge of the cell. Paxillin is a structural protein that allows numerous simultaneous interactions, which are further modified by phosphorylation (Turner 2000). Interestingly, paxillin may also link talin to the cytoplasmic tail of α integrins, thereby increasing the stability of the integrin–talin–actin interaction (Alon et al. 2005). Tensin is recruited to FAs at a later stage than paxillin and couples integrins to actin (Zamir and Geiger 2001). Tensin also interfaces with signaling pathways through binding tyrosine-phosphorylated FAK, p130Cas, epidermal growth factor receptor (EGFR), the Akt kinase PDK1, and the RhoGAPs (Legate et al. 2009).

Integrins Play a Role in Actin Polymerization and Cytoskeletal Dynamics

The physical link between integrins and actin is required for local regulation of actin polymerization as well as for global control of cytoskeletal dynamics. The underlying mechanisms are not fully understood; however isolated integrin adhesions contain the complete machinery necessary for actin polymerization (Butler et al. 2006). This includes the Arp2/3 complex, which controls the assembly of a branched actin filament network in the lamellipodium through its actin nucleation function, and the Rho GTPases, which play key roles in the global regulation of actin dynamics.

Arp2/3 complex

The Arp2/3 complex is a seven-subunit protein that plays a major role in regulation of the actin cytoskeleton by stimulating actin polymerization. Arp2/3 complex has no endogenous actin nucleating activity and must be activated by the Wiskott–Aldrich Syndrome protein (WASP)/Scar family of activator proteins (Pollard 2007). Arp2/3 is recruited to nascent integrin adhesions through interactions with FAK and vinculin to promote actin polymerization, which in turn generates the protrusive force for the lamellipodium. FAK and vinculin are required for normal lamellipodium formation.
Rho GTPases

Rho GTPases play key roles in the global regulation of actin dynamics. The Rho GTPases are a family of ~20 signaling proteins that cycle between an active GTP-bound form and an inactive GDP-bound form (Jaffe and Hall 2005). Cycling is regulated by three sets of proteins: guanine nucleotide exchange factors (GEFs), GTPase-activating proteins (GAPs), and guanine nucleotide dissociation inhibitors (GDIs). In addition to regulating actin dynamics Rho GTPase proteins also play a role in cell proliferation, apoptosis, gene expression, and other multiple common cellular functions. Recruitment and activation of GEFs and GAPs at FAs regulate and control the abilities of Rho GTPases to establish cell polarity and directional migration (Jaffe and Hall 2005). The most important GTPases for the regulation of actin dynamics at FAs are Rac, Cdc42, and RhoA, which are described below.

Rac

The small GTPase, Rac, is thought to be a principal regulator of lamellipodium formation. Ligation and clustering of integrins leads to membrane targeting of Rac and recruitment of multiple GEFs via signaling complexes such as the FAK–p130Cas–Crk–DOCK180 pathway and the paxillin–GIT–PIX pathway. Rac can regulate actin polymerization by activating the Arp2/3 complex via the Scar/WAVE proteins. Rac also increases the availability of free actin-barbed ends by the removal of capping proteins as well as increases the availability of actin monomers by regulating coflin (Jaffe and Hall 2005). Rac also feeds back to integrins by promoting recruitment and clustering of activated integrins at the edge of the lamellipodium (Kiosses et al. 2001).

Cdc42

Cdc42 (cell division cycle 42) is a cell cycle protein primarily thought of as a regulator of cell polarity in epithelial cells and neurons but not in mesenchymal cells. Cdc42 activity has been shown to be regulated by Src activity and ligand binding to integrins can activate Cdc42 via the ILK–Pinch–parvin complex (Legate et al. 2006). Cdc42 regulates polarity by directly binding WASP, which then activates Arp2/3 and subsequent lamellipodium formation, as well as by orienting the microtubule-organizing center and Golgi apparatus in front of the nucleus, toward the leading edge (Jaffe and Hall 2005). It is thought that Cdc42-induced filopodia act as precursors for Rac-induced lamellipodia, establishing a temporal hierarchy of integrin-dependent Rho GTPase activation in the regulation of cell spreading and migration (Guillou et al. 2008).

RhoA

RhoA is another small GTPase which activates two key effector pathways and is primarily thought of as a regulator of cell contractility. RhoA activates the Rho kinase pathway, which acts downstream to activate ROCK and promote contractility through
the phosphorylation of the regulatory light chain of myosin II. RhoA also activates the diaphanous-related forming (DRF) protein family, which regulates actin bundling and microtubule stability (Fukata et al. 2003). Thus initial phases of cell adhesion or lamellipodial protrusion require suppression of RhoA activity and thus RhoA is activated only when contraction is required to retract the trailing edge of cells. Activation and inactivation of RhoA is regulated by integrin-dependent signaling.

Integrins Signal from the Outside-In and Crosstalk with Growth Factors to Control Downstream Intracellular-Signaling Pathways

Integrins are unique, for they can signal from localized stimuli captured from their interactions with ECM as well as integrate mechanical and chemical signals due to their direct association with the cytoskeleton. Furthermore, integrins are able to crosstalk with growth factor receptors and integrins are in fact required for many growth factor receptors to function (Legate et al. 2009). For example growth factor stimulation and integrin-mediated adhesion together increase the intensity and duration of extracellular signal-regulated kinase (ERK) activation. Furthermore phosphorylation of the epidermal growth factor (EGF) receptor is altered more when activated by cell adhesion compared to when stimulated by EGF ligand alone. This implies that integrin activation induces clustering of growth factor receptors including the EGF-, platelet-derived growth factor (PDGF)-, and fibroblast growth factor (FGF)-receptors.

Signaling pathways activated downstream of the integrins are extremely complicated and differ depending on the cell types and physiologic conditions. Nevertheless some key molecules are recognized to be important in the formation of signaling complexes that initiate transduction of integrin-dependent signaling.

Src–FAK-Signaling Complex

One of the key complexes required to initiate integrin-dependent signaling is the Src–FAK complex. Src is a nonreceptor tyrosine kinase constitutively associated with the cytoplasmic tail of β3 integrins via its SH3 domains (Harburger and Calderwood 2009). Src is a member of the src kinase family, which also includes the ubiquitously expressed Src, Fyn, and Yes proteins. All Src proteins are regulated by a C-terminal tyrosine residue that, when phosphorylated, binds to the SH2 domain to autoinhibit kinase activity. Clustering of integrins induces Src activation. The nonreceptor tyrosine kinase FAK is activated by integrin ligation to induce autophosphorylation on tyrosine residue 397. This induces an interaction with Src that stabilizes the active conformation of Src, leading to increased catalytic activity. Subsequently, additional tyrosines on FAK are phosphorylated, resulting in full activation of both kinases (Schlaepfer and Hunter 1996; Calalb et al. 1995). FAK can also be regulated by the receptors for growth factors including EGF and FGF.
Thus FAK also serves as an important intersection point for integrins and growth factor receptors. (Parsons 2003).

**Ras–MEK–MAPK-Signaling Pathway**

A major signaling pathway downstream from the Src–FAK complex is the Ras–MEK–MAPK-pathway, which is activated by integrated signals from integrins and growth factors. Phosphorylation of the downstream kinases, MAPK and ERK2, modulates the dynamics of the focal adhesions as well as cell proliferation, cell cycle progression, and survival (Walker et al. 2005). Integrin signaling can influence MAPK activation on multiple levels. The Src–FAK complex can activate PAK1, which in turn phosphorylates and activates MEK1. MEK1 is required for adhesion-mediated signaling to proceed to MAPK activation and thus serves as a critical convergence point between growth factor and integrin signaling (Slack-Davis et al. 2003). Another intersection point of these two pathways occurs at the level of Raf1. Thus there are multiple ways by which the Src–FAK complex regulates ERK and this regulation is cell-type-specific since deletion of FAK leads to proliferation defects in mammary epithelial cells (Nagy et al. 2007) and cardiomyocytes (Peng et al. 2006) but not in keratinocytes (Schober et al. 2007) or endothelial cells (Braren et al. 2006).

**Akt/PI3K-Signaling Pathway**

Another serine/threonine kinase that is often activated downstream of integrins is Akt, which is activated in a PI-3-kinase dependent manner. FAK activation leads to PI-3-kinase recruitment to focal adhesion resulting in elevation of local PtdIns-3,4,5-P3 levels which serve to catalyze various signaling reactions. Activation of PI-3-kinase leads to downstream Akt activation, which regulates integrin-mediated cell survival. Akt is initially activated by phosphorylation of Thr308 in the activation loop after which it is phosphorylated on different threonine and serine residues by other kinases such as mTOR. ILK may also represent a potential intersection point for growth factor receptor and integrin signaling as ILK is connected to growth factor receptors via a Nck2–PINCH–ILK interaction (Vaynberg et al. 2005; Velyvis et al. 2003) and ILK is connected to integrins and the cytoskeleton as described above.

In summary, integrin ligation leads to the activation of multiple signaling cascades with well-characterized outputs. Activation of these cascades occurs through multiple pathways which are spatiotemporally regulated, both through the assembly of signaling modules as well as through regulation of plasma membrane order and endocytosis. The question of how these processes are regulated in three-dimensional environments as well as in vivo requires detailed exploration. The use of real-time imaging to study the assembly, activity, and targeting of signaling complexes might provide valuable insights.
Perspective

In this chapter we have discussed some of the general features of the structure and function of the integrin family of receptors. Over the past 20 years, a vast amount of information has been discovered with regards to integrins and the crucial roles they play in biological cellular functions. Gene technology has allowed the ability to generate integrin knockout mice which have provided important insights into the functions of the various integrin heterodimers. We have highlighted the emergence of important integrin structural data which has helped elucidate some of the pathways by which integrins undergo conformational changes to become activated and attain the ability to signal bidirectionally. We have also highlighted how integrins are critically involved in extracellular matrix assembly and cell migration. Finally, we have provided a framework to show how integrins modulate many intracellular signaling cascades.

References


Integrin Structure and Function


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