Cell Junctions

Adhesion, Development, and Disease

Edited by
Susan E. LaFlamme and Andrew Kowalczyk

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The Editors

Prof. Dr. Susan E. LaFlamme
Albany Medical College
Center for Cell Biology and Cancer Research
47 New Scotland Avenue
Albany, NY 12208
USA

Prof. Dr. Andrew Kowalczyk
Emory University
Departments of Cell Biology
and Dermatology
615 Michael Street
Atlanta, GA 30322
USA

Cover
Cell layers of the epidermis: The epidermis connects to the underlying dermis via a specialized basement membrane (orange layer). Desmosomes (red dots) and hemidesmosomes (blue dots) serve as anchorage points for cytokeratin intermediate filaments (green lines) to the plasma membrane. (Courtesy of Peter J. Koch and Ansgar Schmidt, see Chapter 12)

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Preface

The evolutionary appearance of multicellular organisms is coincident with the emergence of molecular mechanisms that allow cells to contact their neighbors and to receive positional cues essential for the development of complex tissue architecture and function. Cells interact with one another and with the extracellular matrix (ECM) through a variety of mechanisms. Often, large macromolecular complexes assemble at sites of contact in order to perform specific functions, including adhesion, communication, and to form barriers between tissue compartments. These complex molecular assemblies – or "cell junctions" – are now thought of as cellular organelles that not only mediate physical interactions, but also couple cell contact to signaling pathways that influence cell shape, cell-cycle progression, and gene expression. The manifestation of these various functions becomes apparent in model organisms in which genes encoding cell junction proteins have been ablated, and in the growing list of human disorders that are now known to result from gene mutations in junction components. Indeed, the phenotypes and clinical presentations of these genetic and acquired disorders are impressive in both scope and variety.

The chapters of this book cover a wide range of cell–cell and cell–matrix interactions. The first section of the book focuses on cell–matrix interactions, including focal adhesions and hemidesmosomes. Embedded within these chapters are discussions of integrin activation and cytoskeletal interactions, as well as signaling mediated by integrins and their cytoplasmic binding partners. The importance of the physical three-dimensional structure of the ECM in regulating cell behavior is underscored. The second section of the book concentrates on cell–cell interactions, including anchoring junctions such as adherens junctions and desmosomes, which utilize cadherins as the major adhesive molecules. Tight junctions and gap junctions are also highlighted, with unique insights provided into the roles of these proteins in complex epithelia and disease pathogenesis. Several chapters in the volume converge on the regulation of cell junction dynamics, including the turnover of membrane proteins, the exchange of components into and out of junctions, and the interface between membrane-trafficking pathways and adhesion receptors. The book concludes with a chapter on how cell–cell and cell–matrix interactions are coordinated during complex morphogenic processes.

We hope that the assembly of these chapters – all in one bound volume and authored by some of the world's leading investigators – provides a resource that is valuable to the new student of cell junctions, to experienced scientists in the field, and to those educators who require a comprehensive assembly of overviews in the discipline of cell contact and adhesion. We are enormously grateful to the authors of each chapter for their outstanding contributions. It is our hope that this book provides not only a resource and compilation of existing knowledge and insight, but also a foundation for future studies designed to understand how cell contact and adhesion influence development and human disease.

Susan E. LaFlamme
Albany Medical College
Albany, NY
USA

Andrew Kowalczyk
Emory University
Atlanta, GA
USA

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Contents

List of Contributors

Panos Z. Anastasiadis
Mayo Clinic
Department of Cancer Biology
4500 San Pablo Road
Jacksonville, FL 32224
USA

Asoka Banno
University of California, San Diego
Department of Medicine
9500 Gilman Drive, MC 0726
San Diego, CA 92093-0726
USA

Keefe T. Chan
University of Wisconsin-Madison
Department of Molecular and Cellular Pharmacology
1550 Linden Drive
Madison, WI 53706
USA

Christine M. Chiasson
Emory University School of Medicine
Departments of Cell Biology and Dermatology
615 Michael Street
Atlanta
GA 30322
USA

Christa L. Cortesio
University of Wisconsin-Madison
Department of Biomolecular Chemistry
1550 Linden Drive
Madison, WI 53706
USA

C. Michael DiPersio
Albany Medical College
Center for Cell Biology and Cancer Research
47 New Scotland Avenue
Albany, NY 12208
USA

Deana M. Ferreri
Albany Medical College
Center for Cardiovascular Sciences
47 New Scotland Avenue
Albany, NY 12208
USA

Mark H. Ginsberg
University of California, San Diego
Department of Medicine
9500 Gilman Drive
San Diego, CA 92093-0726
USA

Cara J. Gottardi
Northwestern University
Feinberg School of Medicine
Department of Medicine
Division of Pulmonary and Critical Care
240 East Huron St.
Chicago, IL 60611
USA

Jun-Lin Guan
University of Michigan Medical School
Department of Internal Medicine
Division of Molecular Medicine and Genetics
and
Department of Cell and Developmental Biology
Ann Arbor, MI 48109-2200
USA

Kevin Hamill
Northwestern University
Feinberg School of Medicine
Department of Cell and Molecular Biology
303 E. Chicago Avenue
Chicago, IL 60611
USA

Anna Huttenlocher
University of Wisconsin-Medison
1550 Linden Drive
Madison, WI 53706
USA

Jonathan C.R. Jones
Northwestern University
Feinberg School of Medicine
Department of Cell and Molecular Biology
303 E. Chicago Avenue
Chicago, IL 60611
USA

Patricia J. Keely
University of Wisconsin
Department of Pharmacology
Paul C. Carbone Comprehensive Cancer Center
1300 University Ave
Madison, WI 53704
USA

Kristina Kligys
Northwestern University
Feinberg School of Medicine
Department of Cell and Molecular Biology
303 E. Chicago Avenue
Chicago, IL 60611
USA

Peter J. Koch
University of Colorado at Denver and Health Science Center
Department of Dermatology
12800 East 19th Avenue
Aurora, CO 80045
USA

Michael Koval
Emory University School of Medicine
Department of Medicine
Division of Pulmonary, Allergy and Critical Care Medicine
Atlanta, GA 30322
USA

Andrew P. Kowalczyk
Emory University School of Medicine
Departments of Cell Biology and Dermatology
615 Michael Street
Atlanta, GA 30322
USA

Susan E. LaFlamme
Albany Medical College
Center for Cell Biology and Cancer Research
47 New Scotland Avenue
Albany, NY 12208
USA

Laura J. Lewis-Tuffin
Mayo Clinic
Department of Cancer Biology
4500 San Pablo Road
Jacksonville, FL 32224
USA

Carien M. Niessen
University of Cologne
Center for Molecular Medicine
Joseph Stelzmannstrasse 9
50931 Köln
Germany

Ansgar Schmidt
Philipps-University of Marburg Medical School
Institute of Pathology
Baldingerstrasse
35033 Marburg
Germany

Feng Shi
University of Rochester School of Medicine and Dentistry
Aab Cardiovascular Research Institute
221 Bailey Road
West Henrietta, NY 14586
USA

Jane Sottile
University of Rochester School of Medicine and Dentistry
Aab Cardiovascular Research Institute
221 Bailey Road
West Henrietta, NY 14586
USA

David A. Tumbarello
Cambridge Research Institute
Li Ka Shing Centre
Robinson Way
Cambridge
CB2 0RE
UK

Christopher E. Turner
SUNY Upstate Medical University
Department of Cell and Developmental Biology
750 East Adams St.
Syracuse, NY 13210
USA

Peter A. Vincent
Albany Medical College
Center for Cardiovascular Sciences
47 New Scotland Avenue
Albany, NY 12208
USA

Youngdong Yoo
University of Michigan Medical School
Department of Internal Medicine
Division of Molecular Medicine and Genetics
and
Department of Cell and Developmental Biology
Ann Arbor, MI 48109-2200
USA

Part One Cell–Matrix Junctions

1
The Ins and Outs of Integrin Signaling

Asoka Banno and Mark H. Ginsberg

1.1
General Overview

1.1.1
Integrin Receptors

Integrins are adhesion receptors found in the metazoa, from the simplest sponges and cnidaria to the most complex mammals [1]. They are glycosylated heterodimers composed of non-covalently associated type I transmembrane α and β subunits [2–4]. Each integrin subunit contains a large extracellular domain (N-terminus of >700 residues), a single transmembrane domain (>20 residues), and a generally short cytoplasmic domain (C-terminus of 13–70 residues). Integrin transmembrane domains are thought to begin after an extracellular proline residue, whereas the boundary between the transmembrane and the cytoplasmic domains is less clear [5]. Membrane-proximal regions of the cytoplasmic tails are well conserved in most human integrin α and β subunits, and the boundary between the transmembrane and cytoplasmic domains is predominantly assumed to lie between the conserved W/Y and K/R residues [6, 7]. This conserved K/R residue is usually followed by a stretch of four to six apolar residues, resulting in the K/R residue being flanked by hydrophobic regions [6, 7]. The conserved sequences following K/R residues are GFFKR in α subunits and LLxxxHDRRE in β subunits [6, 7]. These apolar residues are in turn followed by a strongly polar sequence.

In humans, 18 α and eight β subunits have been identified, and these can form at least 24 different heterodimers. In general, each of the 24 integrins has a distinct, non-redundant function, binding to a specific repertoire of cell surface, extracellular matrix (ECM), and soluble protein ligands [1]. The specificity of integrin function is mainly determined by the combination of α and β subunits in each integrin, and by the cellular repertoire of the integrin expression and the functional state of those integrins, in combination with the availability of specific integrin ligands [1, 4]. In addition, the same integrin molecule can have differing ligand specificity, depending on the cell type on which they are found [8]. Further-

more, alternative splicing of extracellular and cytoplasmic domains adds to the potential complexity of integrin structure and function [2, 4, 9].

Since the recognition of the integrin family and the introduction of the term "integrin" about 20 years ago, they have been studied extensively and have become relatively well understood adhesion receptors [1, 10]. Playing central roles in cell migration and cell–ECM adhesions and controlling cell differentiation, proliferation, and apoptosis, integrins are an essential contributor in development, immune responses, leukocyte trafficking, hemostasis, and numerous human diseases such as cancer and autoimmune diseases [1, 2].

1.1.2 Functions

Cell attachment and responses to the ECM are an important requirement for the development of a multicellular organism. Integrins serve as transmembrane mechanical links between the ECM outside of a cell and the cytoskeleton inside of the cell [1, 11]. In this way, they connect ECM ligands (e.g., fibronectin, vitronectin, collagen, laminin) to actin microfilaments [1, 8, 10, 12, 13] or other cytoskeletons. Integrin–ECM interaction leads to the occupancy and clustering of integrins, which in turn promotes the recruitment of cytoskeletal and cytoplasmic proteins such as talin, paxillin, and α-actinin to form focal complexes and focal adhesions [4]. Focal complexes and focal adhesions are dynamic in that they are continually assembled, disassembled, and translocated during cell proliferation, spreading, polarization, and migration [14]. Within adhesion complexes, the cytoplasmic domains of the clustered integrins recruit cytoskeletal proteins and signaling molecules into a close proximity at high concentrations, enabling integrins to initiate intracellular signaling cascades. These signaling events enable integrins to regulate a variety of cellular behaviors [15, 16]. This process is referred to as "outside-in" integrin signaling, and results in the activation of protein tyrosine kinases such as focal adhesion kinase (FAK), Src-family kinases, and Abl, and a serine-threonine kinase such as AKT (PKB) [16, 17]. As an integral part of plasma membrane, connecting the cytoskeleton to the ECM, integrins play a role in mechanotransduction and mediate the transmission of mechanical stress across the plasma membrane [18, 19]. They are also capable of transducing physical forces to chemical signals into the cell, with a help of other signaling molecules that colocalize in focal adhesions [18, 20]. Changes in the balance of forces across integrins and resulting alternations in cell shape induce cells to switch between growth, differentiation, and apoptosis [19].

Many integrins are expressed and remain in the low-affinity binding state until cellular stimulation transforms them into a high-affinity form. Cells can use this transformation to modify their adhesive properties by varying the specificity and affinity of a given integrin [9, 15]. This regulation of cell adhesion by signaling from within the cell is referred to as "inside-out" signaling [1, 5, 9]. Rapid changes in integrin-mediated adhesion are often precisely regulated in time and space in biological settings such as platelet aggregation and leukocyte transmigration [9,

21]. Although "inside-out" signaling can be achieved via the modulation of integrin affinity for the ligand, or via affinity-independent mechanisms such as changes in integrin diffusion and integrin clustering, a major focus of integrin research over the past decade has been on the affinity-dependent regulation of integrin-mediated adhesion, a process that will be operationally defined here as integrin activation [1, 5, 9, 15]. Efforts at deciphering the mechanism of this form of "inside-out" signaling have produced compelling evidence that the integrin cytoplasmic domains are the targets of intracellular signals that modulate ligand binding affinity [21, 22].

As noted above, integrins are involved and play essential roles in many biological processes, and many excellent reviews have discussed how signals from these receptors regulate cellular behaviors [4, 11, 12, 14, 16, 20, 23]. Here, attention will be focused on how cells can change integrin affinity in response to developmental events or to changes in their environment, a process that is widely used in biological functions such as cell adhesion, cell migration, cellular aggregation, and leukocyte transmigration during inflammation.

1.2 Integrin Activation

1.2.1 Definition

Under physiological conditions, many integrins are in equilibrium between low- and high-affinity states [1]. The rapid process of shifting from the low-affinity state to the high-affinity state of integrins will be operationally defined here as "integrin activation". Activation – that is, the process of becoming active – is a term that requires definition depending on context. For example, in the case of many signaling receptors, "activation" refers to the occupancy of that receptor by an extracellular ligand with transmission of signaling events into the cell. Since integrins can also perform this function ("outside-in" signaling), the term "integrin activation" is sometimes encountered when referring to intracellular signals that result from integrin occupancy [6, 24]. Therefore, in order to avoid any confusion, the clear definition of the term "activation" with respect to the meaning of what is the "active" state of the integrin is recommended. Here, the term "activation" is used when referring to transition to the high-affinity state, an event that can be accomplished by the propagation of conformational changes from the integrin cytoplasmic domains to the extracellular domains [1, 5, 25, 26].

Activation and inactivation of integrins are tightly regulated. Although not all integrins have been shown to undergo extremes of activity, it is generally believed that most integrins shift between active and inactive states in a localized fashion when it is important for cells to regulate their adhesion in a temporal and spatial manner [1, 27]. The importance of integrin activation is well demonstrated in the functions of platelets and leukocytes [1, 9].

1.2.1.1 Platelets

αIIbβ3 integrins, also known as GPIIb-IIIa, are present at high density on resting circulating platelets but remain inactive under normal conditions [1, 9]. Upon platelet stimulation by agonists such as thrombin, ADP, or epinephrine acting through G protein-coupled receptors, or by von Willebrand factor signaling through its receptor (GPIb/V/IX), or by collagen binding to its receptor, GPVI, signals from within the cell activate αIIbβ3 integrin to bind to ligands such as fibrinogen, von Willebrand factor, and fibronectin [1, 9]. Binding of these multivalent ligands to activated αIIbβ3 leads to platelet aggregation. It is crucial that αIIbβ3 is inactive on resting circulating platelets, for if it were constitutively active or if its activation were to become deregulated, then unregulated platelet aggregation could lead to thrombosis [1, 9]. In contrast, defects in or a lack of αIIbβ3 integrins result in defects in hemostasis, as seen in a bleeding disorder known as Glanzmann thrombasthenia [1].

1.2.1.2 Leukocytes

β2 integrins, also known as CD11/18, on leukocytes are regulated in a very similar fashion to αIIbβ3 integrins on platelets [1, 9]. They are expressed on most white blood cells in their resting state. When the cells encounter agonists such as chemokines, the β2 integrins are rapidly activated to mediate firm adhesion to the integrins' ligands. Their ligands include counter-receptors such as Ig superfamily molecules (e.g., ICAM-1, 2, and 3), ECM proteins (e.g., fibronectin), blood-clotting proteins such as fibrinogen, and the complement pathway product, C3bi [1, 28, 29]. Intracellular adhesion molecules (ICAMs), which are major ligands of β2 integrins, are expressed on cells to allow the attachment of leukocytes to the cells. The β2 integrin–ICAM interaction mediates processes such as phagocytosis, cytotoxic killing, and efficient antigen presentation [1, 28]. Further, like αIIbβ3 integrins on platelets, it is critical that β2 integrins remain inactive on resting leukocytes, for deregulated activation or impaired deactivation of β2 integrins could cause failure in normal immune responses [30]. Similarly, a lack of or dysfunction in rapid activation of β2 integrins leads to the defective immune function seen in patients with leukocyte adhesion deficiency (LAD) who suffer from leukocytosis and the failure to recruit leukocytes to sites of infection [1, 9].

1.2.2 Structural Basis of Activation

As noted above, integrin activation is usually due primarily to conformational changes in the extracellular domains of the integrins. Because these conformational changes are believed to initiate at the cytoplasmic face of the integrin, they must somehow traverse the plasma membrane. Several key advances have provided important new insights into each step in the activation process: namely, the nature of the conformational change in the extracellular domain; the mechanism of transmission across the membrane; and how a specific protein – talin – interacting with the β cytoplasmic domain causes these long-range allosteric rearrangements.

1.2.2.1 Extracellular Rearrangements

Based on studies using crystallography, nuclear magnetic resonance (NMR), electron microscopy, and Förster resonance energy transfer (FRET), integrins appear to assume at least three conformations: (i) a bent "closed" conformation; (ii) an intermediate extended conformation with a closed headpiece; and (iii) an extended "open" conformation [3, 25, 31, 32]. Several studies have suggested that the bent conformation is a low-affinity state, whereas the extended conformations are associated with the high-affinity state of integrins [31–37]. However, the bent form can also bind ligand with high affinity [38] in some circumstances, and has led research workers to propose an alternative "deadbolt" model for "inside-out" activation, emphasizing that extended conformation is not necessary for integrins to bind to their physiological ligands [38, 39]. Likewise, the results of one study suggest that the extension of integrins may be regulated by different signaling pathways in a temporally specific manner rather than being a fundamental requirement for integrin activation [40]. Thus, it is very likely that integrins are in dynamic equilibrium among different conformational states [24, 32, 35, 41], and the global rearrangements in integrin conformation accompanying "inside-out" signaling remain incompletely understood [35, 42, 43].

In addition to the modulation of integrin affinity via conformational change within a single receptor molecule (affinity modulation), increased integrin-mediated adhesion can also be caused by receptor clustering on the cell surface (avidity modulation) [44–47]. Furthermore, in most circumstances it is likely that some combination of conformational change and receptor clustering is involved in the regulation of integrin-mediated adhesion. In one perceptive report, a monovalent antibody and a conditional dimerizer were used to isolate the relative contributions of affinity modulation and receptor clustering in αIIbβ3-mediated functions [48]. In particular, these studies showed that affinity modulation and avidity modulation play complementary roles in the adhesive functions of this integrin.

1.2.2.2 Transmembrane Propagation

Integrin activation by "inside-out" signaling must involve the transmission of conformational rearrangements from the cytoplasmic domains to the extracellular domains; therefore it would be expected that the transmembrane domain of integrins is involved in the signal transduction process. These regions are highly conserved amongst each of the integrin α- and β-subunit families, and are also conserved between species [49]; indeed, mutations in this region can lead to a loss of integrin expression [50–52]. In fact, recent studies have begun to provide insight into how rearrangements within the transmembrane domain can lead to integrin activation.

Mutational analysis and molecular, computational modeling together suggested that a helical interface between the integrin α and β subunit transmembrane domains stabilizes the inactive state, and also suggest that disruption of this helical transmembrane interface leads to activation [50, 53–55]. To date, four models have been proposed to explain the mechanism underlying the proposed rearrangements in transmembrane domain and the sequential disruption of the α-β trans-

membrane interface: separation, pistoning, twisting, and hinging [1, 6, 7]. These all involve some changes in the orientation of the subunits relative to one another and to the membrane. Although recent findings support the separation and pistoning models, high-resolution structures of the transmembrane domains will be required to distinguish clearly between the different activation models [5, 6, 31].

The helical packing of integrin transmembrane regions is likely to depend on specific crossing angles and specific in-register side-chain arrays [55]. Furthermore, the insertion of the integrin membrane-proximal domain can vary, either shortening or lengthening the transmembrane domain and changing the number of residues buried within the lipid bilayer [3, 5, 49, 55]. Based on these data, the pistoning model suggests that intracellular activating signals could shorten the transmembrane helix, in response to which its membrane tilt angle and register with the neighboring helix change in order to avoid hydrophobic mismatch with the fixed width of the membrane bilayer. This helical mismatch may be the critical event in disruption of transmembrane interactions that stabilize the low-affinity conformation, leading to integrin activation [3, 5, 55]. This model is supported by a report showing that the majority of mutations identified by random mutagenesis throughout the transmembrane domain activated integrins most likely by disrupting or shortening the transmembrane helix [3, 55]. Changes in the length or orientation of the integrin transmembrane domain may also take place during physiological integrin activation [5].

1.2.2.3 **Intracellular Rearrangements**

Changes in interactions and/or in the structures of the cytoplasmic domains of integrins within the highly conserved regions play crucial roles in integrin activation via "inside-out" signaling [5–7, 56]. Indeed, there is direct experimental evidence for a change in the relationship of the integrin α and β cytoplasmic domains during integrin activation [25].

The interaction between the membrane-proximal regions of the α and β subunits is believed to occur in part through a salt bridge between a conserved Arg in the α tail and an Asp in the β tail and the hydrophobic residues immediately N-terminal to the Arg and Asp [21, 56, 57]. Indeed, this specific association between the α and β subunits is thought to prevent integrin activation by stabilizing the low-affinity state [2, 5, 26]. Mutations that disrupt this "clasp" lead to integrin activation [21, 22, 26, 56]. Integrins can be constitutively activated by deletion in the entire α subunit cytoplasmic tail or of the membrane-proximal GFFKR sequence [21, 22, 26, 56]. Deletion or certain point mutations in the membrane-proximal region of the β tail also result in integrin activation [26, 56, 58]. Furthermore, replacement of the cytoplasmic–transmembrane regions by heterodimeric coiled-coil peptides or an artificial linkage of the tails inactivates the receptor, and breakage of the coiled-coil or clasp activates integrins [58–60]. Taken together, these data strongly indicate that this hydrophobic and electrostatic interaction plays an essential role as a stabilizer in the association of the α and β membrane-proximal regions, maintaining the integrins in a low-affinity state [3, 5, 6]. The important role of α and β tail interaction is further supported by a study using FRET. In the

resting states, although fluorophore-tagged α and β tails were sufficiently close together to undergo FRET, stimulation with agonists or the introduction of activating mutations to membrane-proximal α subunits led to a reduction in FRET [31]. Such reduction was ascribed to separation of the cytoplasmic domains, but could also relate to an alternation in the orientation of α and β cytoplasmic tails relative to each other, without actual separation [3, 31]. In consequence, an important goal for future studies will be to provide a more precise description of these structural rearrangements.

Deletions of mutations in the β tail that are more C-terminal (i.e., in the membrane-distal region) can block integrin activation. In particular, an NPxY/F motif of the β subunit has been identified as one of the critical sites [6, 21, 56, 61]. Tyr/Phe-to-Ala mutations in this conserved motif block integrin activation [6, 62]. It is now known (*vide infra*) that mutations in the NPxY motif perturb the binding of integrin β tails to numerous cytoskeletal and signaling proteins [17], thus accounting for their profound effects on integrin signaling. Hence, membrane-distal portions of the β tail may control integrin activation through interactions with regulatory cytoplasmic proteins and through effects on the conformation of membrane-proximal regions. The physiological integrin activation process also requires the membrane distal region of the β3 cytoplasmic domain [63].

In summary, a current model is that release of the structural constraint upon the cytoplasmic rearrangement initiates a conformational change that propagates across the membrane through rearrangements in the α and β transmembrane domains. This in turn leads to changes in the conformation of the extracellular domains that activate integrins [32, 59]. As noted above, intriguing questions remain at each step of this process, thereby explaining the continued intense research efforts to further clarify this unusual form of signal transduction.

1.2.2.4 **Interactions at the Integrin Cytoplasmic Domains**

The integrin cytoplasmic domains play a central role in integrin activation [21, 22], and overexpression of certain proteins that bind to the cytoplasmic tails can result in integrin activation [21, 22, 58, 59]. The binding of the adaptor, talin, to the β cytoplasmic domain has been identified as a crucial, final step in the activation of several classes of integrins [64–66]. This finding has been confirmed for β1 [67], β2 [31, 68, 69] and β3 [70] integrins; hence, talin appears to play a general role in activating multiple classes of integrins. Moreover, a study using mice harboring point mutations in the β3 cytoplasmic tail provided the first *in-vivo* evidence supporting the importance of talin binding for integrin activation in mammals [71].

A variety of other cellular proteins are reported to interact directly with integrin cytoplasmic tails, and may have roles in integrin activation. β3-endonexin binds specifically to β3 tails through membrane-proximal and -distal motifs, and can activate αIIbβ3 in Chinese hamster ovary (CHO) cells [6, 17]. However, since β3-endonexin-mediated activation depends on the presence of talin, its role may be to cooperate with talin in αIIbβ3 integrin activation in platelets [6, 64]. Cytohesins-1 and -3 bind β2 integrin tails, and their overexpression has been shown to increase adhesion [6, 17]. However, these proteins contain a SEC7 domain and act as

guanine nucleotide exchangers for Arf GTPases. ARF6 can regulate Rac1 activity [72–75], and could therefore increase cell adhesion through affinity-independent processes, such as integrin clustering, rather than integrin activation [6]. Calcium- and integrin-binding protein (CIB) has been proposed to activate αIIbβ3 by binding to the αIIb tail [6, 17]. However, some argue that CIB is involved in post-receptor occupancy events rather than initial activation [6, 17] and a recent, elegant study showed that it blocks integrin activation by inhibiting talin binding [76]. Another α subunit-binding protein, paxillin, plays a key role in cell motility and focal adhesion turnover [77]. In T cells, the α4-paxillin interaction provides resistance to rupture by external shear forces, without changing the integrin affinity of talin [77]. Similarly, while not shown to bind directly to integrin tails, RapL/NORE1B has been reported to associate with αLβ2 and lead to its activation, clustering, and redistribution [78, 79]. Thus, the list of potential integrin activation regulators is long and will keep expanding. It will be of great interest to examine the activators to see if they are talin-independent, function as modulators of talin binding to the integrins, or act cooperatively with talin.

Talin As mentioned above, recent data strongly indicate that talin plays an important role in integrin activation [64–66]. Talin is a major cytoskeletal protein that colocalizes with and binds to integrins, as well as to actin and actin-binding proteins such as vinculin [2, 64–66]. It is an antiparallel homodimer of two ~270 kDa subunits, each of which consists of an N-terminal globular head domain of ~50 kDa and a C-terminal rod domain of ~220 kDa [80, 81]. The head domain contains a FERM domain with three subdomains, F1, F2, and F3, which often mediates interactions with the cytoplasmic tails of transmembrane proteins. The F2 and F3 subdomains of talin bind specifically to integrin β3 tails, although F3 shows a higher affinity than F2 [65]. In addition, the expression of F3 – but not F2 or other high-affinity β tail-binding proteins – activates αIIbβ3 integrins, implying that the major integrin-binding and activating fragment of talin lies within the 96-residue F3 subdomain [2, 65]. Knockdown of talin expression in CHO cells inhibits the activation of both β1 and β3 integrins without altering integrin expression, and this cannot be compensated for by the expression of activating molecules such as activated R-Ras or the CD98 heavy chain [64]. Furthermore, talin knockdown blocks agonist-stimulated fibrinogen binding to the megakaryocyte integrin αIIbβ3, suggesting that normal cellular activation of integrins also requires talin [64].

The talin F3 structure is very similar to that of phosphotyrosine-binding (PTB) domains, which recognize ligands containing β turns formed by NPxY motifs [65]. As discussed above, NPxY motifs are well conserved in most integrin β tails, and mutations that disrupt this motif perturb β turn formation, inhibiting talin binding and therefore interfering with integrin activation [61, 62]. Likewise, mutations within the talin PTB-like domain prevent integrin β tail binding and thereby block integrin activation [64, 82]. These data strongly support the view that the integrin β tail–talin interaction represents a general mechanism for integrin activation.

If talin binding is a final step in integrin activation, then it may be hypothesized that integrin activation is regulated by modulation of the talin binding to integrins. There are two obvious forms of such regulation: (i) alteration of the integrin tails; and (ii) the modification of talin. Tyrosine phosphorylation of the β tail NPxY motif inhibits talin binding, and such modification by Src family kinases inhibits cell adhesion and displaces integrins from talin-rich sites [2, 6]. These data suggest that the phosphorylation of integrins is an important, negative regulator of integrin activation. However, as tyrosine phosphorylation of β3 occurs after αIIbβ3 integrin activation in platelets, it is not clear whether or not such phosphorylation is important in regulating initial integrin activation [2].

The proteolytic cleavage of intact talin releases the head domain and results in an increase in talin binding affinity for the β tail, indicating that the integrin-binding site is masked in intact talin [2, 6, 68]. In fact, in platelets, the protease calpain can cleave talin to release the head domain, implying its role as a regulator of talin-mediated αIIbβ3 activation [2, 6]. However, as calpain also cleaves integrin β tails, resulting in activation blockade, the effects of calpain on integrin activation are complex [2, 6]. Alternatively, the binding of phosphatidylinositol 4,5-biphosphate (PtdInsP2) to talin has also been reported to induce a conformational change, enhancing talin's association with integrin β1 tails [2, 6]. Talin can bind to and also activate a splice variant of the PtdInsP2-producing enzyme phosphatidylinositol phosphate kinase type Iγ-90 (PIPKIγ-90), stimulating PtdInsP2 production, which in turn could promote talin–integrin interactions [2]. Src phosphorylation of PIPKIγ-90 has been said to lead to a dramatic increase in its affinity for talin and to an increase in recruitment to focal adhesions. Since c-Src is activated downstream of integrins, the phosphorylation of PIPKIγ-90 may represent a positive amplification mechanism that links c-Src signaling to integrin activation [2]. Although talin can be phosphorylated, the effects of these phosphorylations on integrin binding and activation remain unclear [2].

Another major question is how talin binding activates integrins. When the ability of activating talin fragments (i.e., F2–F3 domains together or F3 alone) to bind to different regions of the β3 cytoplasmic domain was compared to that of non-activating talin fragment (i.e., F2 domain) by monitoring the perturbation of specific NMR resonances of the β tail, the F2–F3 and F3 fragments – but not F2 – showed distinct perturbation of the membrane-proximal region of the β3 tail, suggesting the involvement of the β3 tail membrane-proximal region in talin-mediated integrin activation [83]. As noted earlier, as the interaction of the α and β membrane-proximal regions maintains the integrins in a low-affinity state [26], it seems likely that perturbations in the region result in integrin activation. NMR studies have suggested that direct disruption of α-β tail interaction by the talin head domain results in integrin activation [59]. In addition, F2–F3 and F3 also perturb the more distal region of the β3 tail [82]. The membrane-distal region of integrin β tail provides a substantial fraction of the binding energy, and has been suggested to contribute to integrin activation [84].

These data together suggest a two-step activation model: the talin head domain first recognizes the high-affinity binding site in the membrane-distal region, which

provides a strong linkage between the talin and the integrin β tail, and subsequently binds to a second lower-affinity membrane proximal site that is involved in α-β association, triggering separation of the tails and integrin activation (Figure 1.1) [82, 85].

There are many other PTB domain-containing proteins that bind to integrin β tails in a similar fashion to talin [82, 86]. Such proteins include Numb, Dok-1, ICAP-1α, and Kindlins [6, 86]. However, talin was unusual in its ability to activate integrins, which led Wegener et al. to reason that there must be additional unique

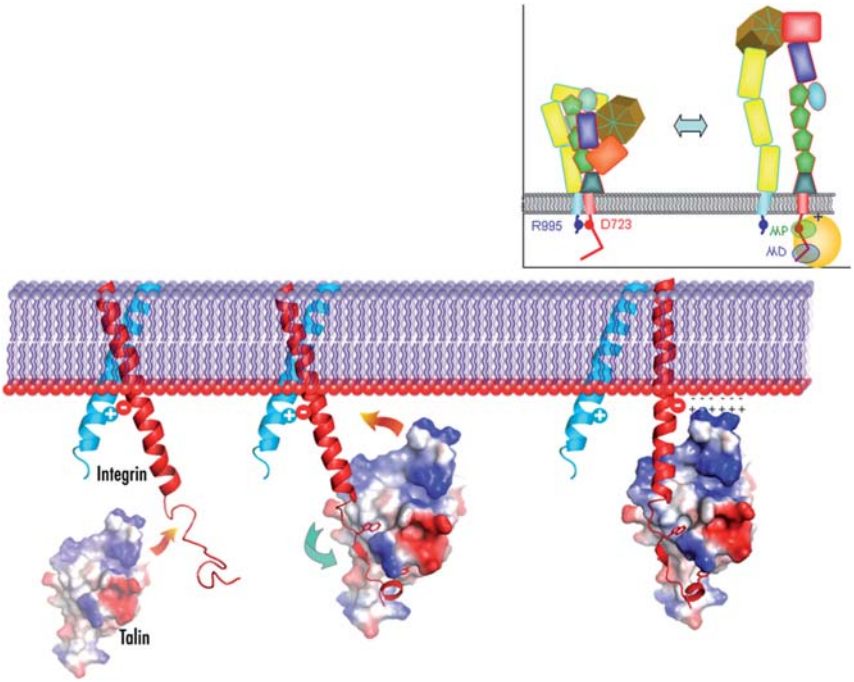

Figure 1.1 Model of talin-induced integrin activation. Left panel: The talin F3 domain (surface representation; colored by charge), freed from its autoinhibitory interactions in the full-length protein, becomes available for binding to the integrin. Center panel: F3 engages the MD part of the β3-integrin tail (red), which becomes ordered, but the α-β integrin interactions that hold the integrin in the low-affinity conformation remain intact. Right panel: In a subsequent step, F3 engages the MP portion of the β3 tail while maintaining its MD interactions. The consequences of this additional interaction are: (1) destabilization of the putative integrin salt bridge; (2) stabilization of the helical structure of the MP region; and (3) electrostatic interactions between F3 and the acidic lipid head groups. The net result is a change in the position of the transmembrane helix, which is continuous with the MP-β-tail helix. This position change causes a packing mismatch with the αIIb-transmembrane helix, separation or reorientation of the integrin tails, and activation (inset). Mutants of F3 that have compromised interactions with the MP region and other PTB domains that lack an MP-binding site stall at point B, consistent with their dominant-negative behavior. (Reprinted from Ref. [85]; © 2007, with permission from Elsevier.)