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Review

Regulation of integrin activity and signalling

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ABSTRACT

The ability of cells to attach to each other and to the extracellular matrix is of pivotal significance for the formation of functional organs and for the distribution of cells in the body. Several molecular families of proteins are involved in adhesion, and recent work has substantially improved our understanding of their structures and functions. Also, these molecules are now being targeted in the fight against disease. However, less is known about how their activity is regulated. It is apparent that among the different classes of adhesion molecules, the integrin family of adhesion receptors is unique in the sense that they constitute a large group of widely distributed receptors, they are unusually complex and most importantly their activities are strictly regulated from the inside of the cell. The activity regulation is achieved by a complex interplay of cytoskeletal proteins, protein kinases, phosphatases, small G proteins and adaptor proteins. Obviously, we are only in the beginning of our understanding of how the integrins function, but already now fascinating details have become apparent. Here, we describe recent progress in the field, concentrating mainly on mechanistical and structural studies of integrin regulation. Due to the large number of articles dealing with integrins, we focus on what we think are the most exciting and rewarding directions of contemporary research on cell adhesion and integrins.

1. Introduction

Research on cell adhesion is one of the most rapidly expanding fields in the biological and biomedical sciences. One reason for this is the realisation that cell adhesion is involved in many essential normal cellular and pathological functions including the formation of cells from malignant tumors and their attachment to secondary organs. Another reason is the fact that recent methodological progress has enabled us to increasingly deepen our understanding of the organisation of complex cellular systems and their regulation. Several excellent reviews have been written on adhesion and on the major molecular families of adhesion molecules. These include the integrins, the cadherins [1], the selectins [2], the adhesion-G protein-coupled receptors [3], the extracellular matrix proteins such as fibronectin [4], collagens, and laminins, and the large immunoglobulin superfamily of adhesion molecules [5,6].

In particular, the integrin family of adhesion molecules is drawing increasing attention. Integrins are fascinating molecules. They are present in all nucleated cells, often in large numbers and many members can be expressed simultaneously in a given cell. They are structurally unusually complex and, importantly, they can act as signalling molecules in both directions across the plasma membrane. Although excellent reviews have been written on integrins including structural and signalling aspects of these molecules [7–12], the field has become more and more difficult to master due to the large amount of published studies on this subject. Therefore, in this review we

eld, how integrin to signal in both directions across the plasma membrane. We have mainly focused on structural aspects of integrin regulation, and how intracellular molecules bind to integrin tails and regulate integrin activity. Although current knowledge is certainly still in its infancy or early youth, we begin to get a glimpse of what integrins look like and how they may function.

Integrins are present in metazoa and sponges, and primitive bilateralia express integrins [8]. For example, *Caenorhabditis elegans* has two integrins, but the number is substantially higher in more developed organisms. In humans there are 24 different integrins, which arise from the noncovalent association between one of each 18 α-subunits and 8 β-subunits (Fig. 1). Importantly, some subunits can combine with several different partners, adding to the structural complexity of integrin receptors. Using knockout mice it has become evident that the integrins possess both redundant and nonredundant functions, and that lack of expression may result in a wide variety of effects ranging from blockage in preimplantation to embryonic or

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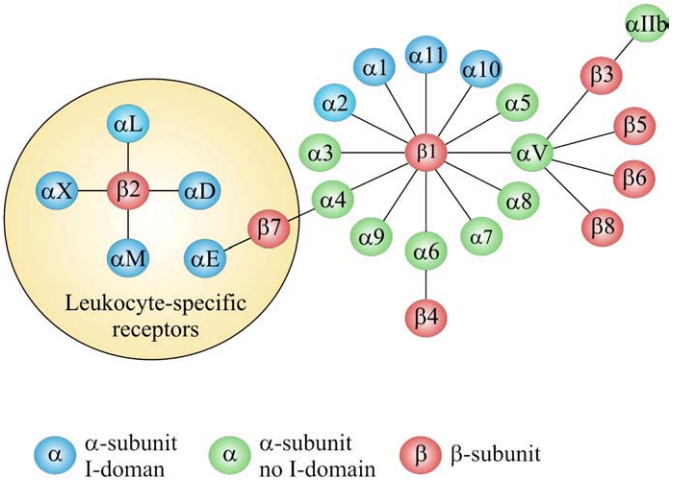

Fig. 1. The integrin superfamily. The integrins can be subdivided according to their β chains but note that some α chains can combine with several β chains. 24 different integrins are present in humans.

perinatal lethality and developmental defects. An excellent example of a natural human knockout is the leukocyte adhesion deficiency syndrome (LAD-I) where mutations in the β_2 integrin chain impair leukocyte functions resulting in severe microbial infections, impaired wound healing, defects in phagocytosis and chemotaxis [13,14].

Integrins are not alone in the plasma membrane. We are just starting to appreciate the fact that often they are part of macromolecular assemblies required for proper signalling. One recent example is the complex between the leukocyte Mac-1 ($\alpha_M\beta_2$) integrin and matrix metalloproteases [15,16]. Interestingly, the β_2 integrin complexes with matrix metalloproteases can be disrupted with peptides, which interfere with the binding between the integrins and the metalloproteases and these peptides efficiently inhibit integrin activity [15,16]. The integrin polypeptides interact on the outside of the cell, but also lateral associations in the transmembrane regions of integrins [17] are important, although less is known about them. Adding to the complexity is the fact that many of these interactions are short-lived and therefore difficult to study.

Integrins communicate over the plasma membrane in both directions and we distinguish between outside-in and inside-out signalling [18,19]. In outside-in signalling through integrins, ligands bind to extracellular integrin domains, where a conformational change occurs so that the signal is transmitted into the cell. Furthermore, also integrin clustering may occur. Inside-out signalling originates from non-integrin cell surface receptors or cytoplasmic molecules and it activates signalling pathways inside the cells, ultimately resulting in the activation/deactivation of integrins. In this case, adhesion may be regulated both by conformational changes in the integrin and by valency change (integrin clustering). In fact, this division into two distinct signalling entities may not be that black and white, instead both signalling events may occur simultaneously and reinforce each other (see below).

In order to understand signalling dynamics, it is absolutely necessary to have a good knowledge of integrin structure. Let us therefore first describe how integrins are constructed.

2. Integrin structure

2.1. The extracellular part of integrins — structural insights

All integrins are type I integral membrane proteins consisting of an α- and a β-subunit forming a heterodimer [20]. In effect, the integrin has a ligand binding "head" on the top of two "legs". Fig. 2A shows a sequence-based schematic drawing of the leukocyte LFA-1 ($\alpha_L\beta_2$, CD11a/CD18) molecule, which may serve as a well studied example. LFA-1 belongs to the integrins which have an inserted (I) domain, also called von Willebrand factor (A) domain in the α chain. In the integrins which have an I-domain (see Fig. 1), this is the primary ligand binding region, whereas in integrins which lack the α chain I-domain, the binding site in the integrin "head" is formed by structural contributions of both the α and β chains [21]. The I-domain is inserted in a G protein-like seven-bladed β-propeller domain [22]. This is followed by an Ig-like "thigh", two β-sandwich "calf", a transmembrane and a cytoplasmic domain. The β polypeptide consists of a PSI (plexin-semaphorin-integrin)-domain, a β I-like domain, an Ig-like hybrid domain, 4 EGF-like domains, a "β-tail", a transmembrane domain and a cytoplasmic tail [21].

The integrins form well-defined domains, and the first integrin I-domain to be crystallised was from Mac-1 (α_M or CD11b) [23] (Fig. 2B, C). The I-domain can exist in two different conformations: an "open" (high affinity) and a "closed" (low affinity) conformation. An important feature is the presence of the "MIDAS" (Metal Ion Dependent Adhesion Site), which coordinates divalent metal cations, required for the integrin high affinity state. This is in agreement with the fact that the first crystal structure attributed to the "open" (high affinity) conformation displayed the MIDAS occupied by a magnesium ion (Fig. 2C), while the structure attributed to the "closed" (low affinity) conformation did not have any cation bound at the MIDAS (Fig. 2B). Nevertheless, subsequent studies led to the conclusion that the MIDAS is likely to be constitutively occupied by a divalent magnesium ion under physiological conditions, and that the metal binding is not correlated *per se* with the transition from closed to open [24].

A remarkable difference between the first two CD11b I-domain structures (Figs. 2B and C) regards the position of the α_7 helix, which in the "closed" conformation is fixed to the central β-sheet (Fig. 2B), while upon activation it is displaced by a downward movement leading to the "open" conformation (Fig. 2C). The metal is now coordinated to six ligands: two serines of a DxSxS motif (x is any amino acid) from the α_1 loop; one threonine from the loop between the α_3 and α_4 helices; two water molecules, of which one is hydrogen bound to two aspartate residues (one from the DxSxS motif, and one from the α_5 loop). The sixth ligand is probably another molecule of water, although in the crystal structure this position is occupied by a glutamate from a neighbouring I-domain [23].

Another interesting structural feature is the β chain I-like domain. Crystallographic studies show that in integrins, which lack the α chain I-domain, the β I-like domain is the primary ligand binding site. It contains three metal binding sites: the MIDAS, the ADMIDAS (adjacent to the MIDAS), and the LIMBS (Ligand Induced Metal Binding Site). In the physiologic low affinity state, all three metal binding sites are occupied: the MIDAS by a magnesium ion, while the other two metal binding sites can be occupied by calcium ions [25]. However, this result is in contrast with previous studies that showed only one metal present in the absence of ligand [26]. The magnesium ion at MIDAS directly coordinates the aspartic acid residue of the RGD ligands (the major binding motif in many integrin ligands), which otherwise would be electrostatically repelled by the anionic residues of the MIDAS itself [25]. Besides its known regulatory role, the ADMIDAS calcium ion can be involved in ligand binding [27].

No complete type I integral membrane proteins have been crystallised so far, and there is no structural information on whole molecules. However, several crystal structures are available for the extracellular portion of type I membrane proteins, produced as recombinant chimeras, or obtained through protease cleavage.

A major breakthrough in integrin research occurred when the external portion of the $\alpha_v\beta_3$ integrin was crystallised in its unbound state. The most surprising fact was that the integrin ligand binding head was turned towards the legs forming a V-like structure (Fig. 2D,

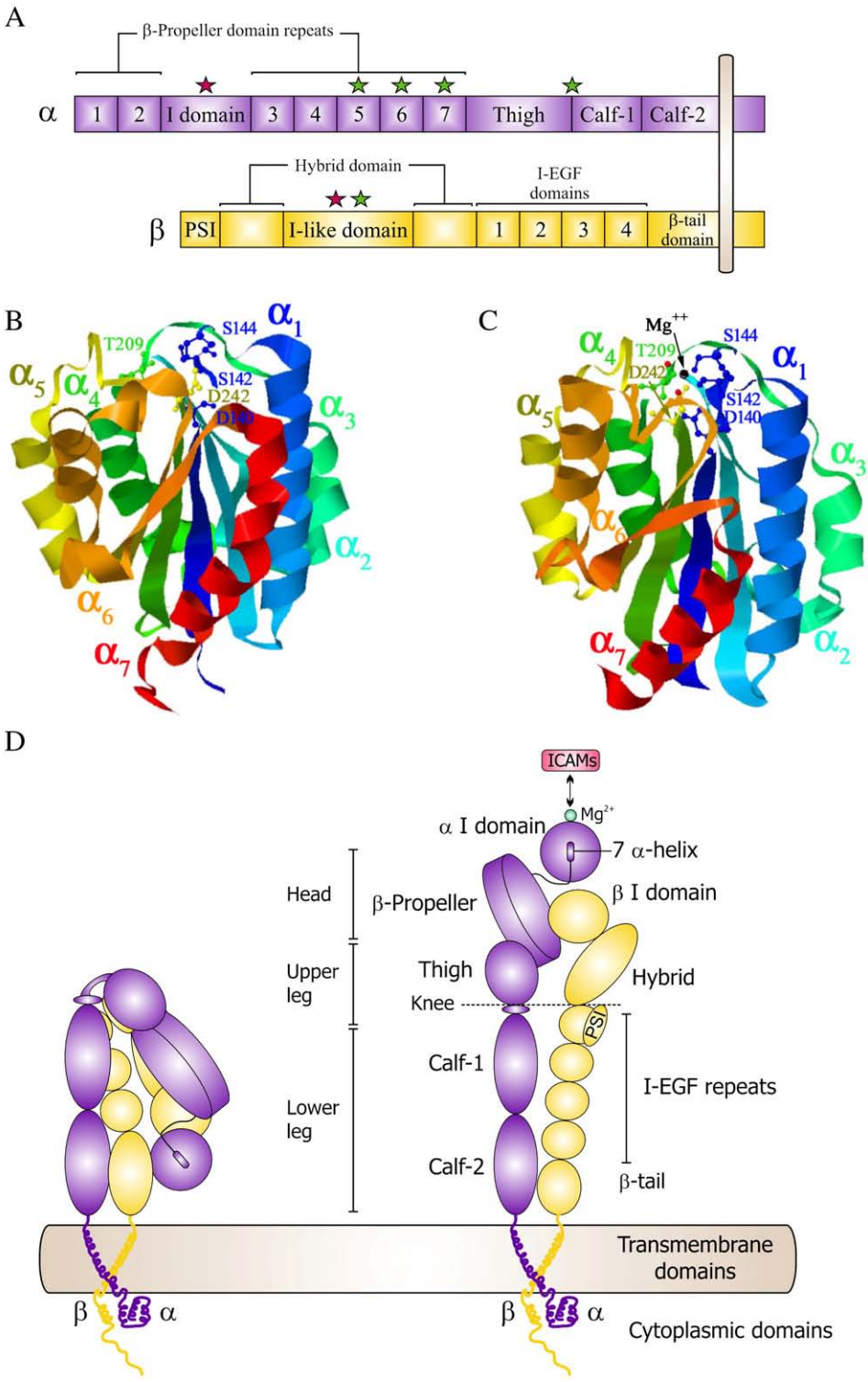

Fig. 2. Structures of integrins and their extracellular domains. A, Schematic drawing of the LFA-1 integrin. Note that the α I-domain is within the β-propeller, and the β I-like domain within the hybrid domain. The positions of the Mg^{+} (★) and Ca^{+} (★) binding sites are indicated; B, Structure of the Mac-1 I-domain in the inactive state. The functionally important α_1 (blue) and α_7 (red) helices are shown. C, Structure of the activated Mac-1 I-domain; note the magnesium ion (black dot) and the shifted position of the α_7 helix. D, Schematic structure of the external portion of $\alpha_V\beta_3$. In the crystal structure, the ligand binding site is turned towards the membrane (left); to the right is shown the stretched-out intermediate affinity form.

left). In the intact integrin, this would mean towards the lipid bilayer [21]. One would envision that such a conformation could make ligand binding more difficult, and for this reason it was proposed that this structure represented the inactive non-bound form, and that in the active form, the integrin would "stand up" to be able to bind ligands (Fig. 2D, right). Whether the integrin head in the active form really is turned towards the membrane has been a matter of controversy [28]. Crystallisation and subsequent soaking with an RGD peptide showed that upon binding, only a minor structural change occurred [26]. The bent configuration was maintained. On the other hand, addition of the same ligand to bent $\alpha_V\beta_3$ in solution induces leg extension and conversion of the headpiece to the open conformation [29]. Work with

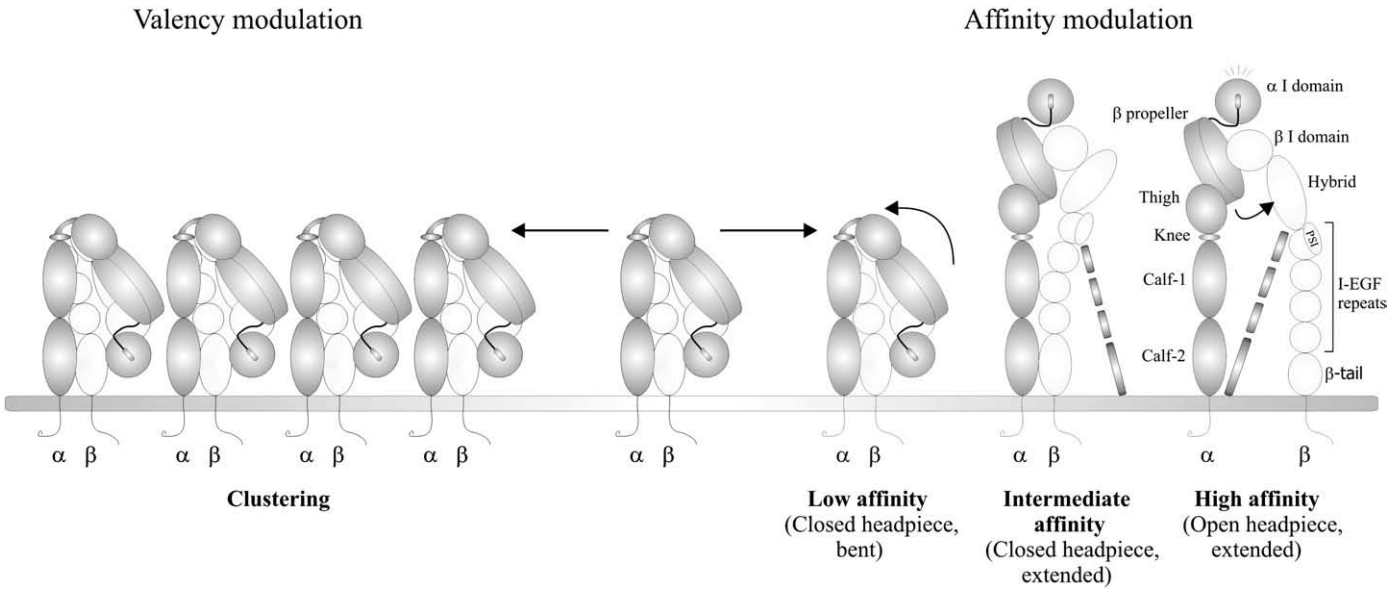

Fig. 3. Valency and affinity modulations of integrins. By clustering of the integrins the *avidity* becomes high enough for functional adhesion (left). An intermediate affinity may be achieved by straightening out of the integrin, but high affinity needs opening of the binding site (right). It should be pointed out that it is not known for sure that the integrins need to straighten out in order to exhibit high affinity.

activating and conformation-specific antibodies also suggests that the β chain is extended in the active integrin, as it can be observed by using the KIM127 intermediate affinity reporting antibody [30].

Using molecular dynamics it was recently reported that when the RGD ligand is replaced by a fibrinogen type III module, and allowed to interact with the integrin headpiece, the hinge angle between the β I-like domain and the hybrid domain in the headpiece of the $\alpha_V\beta_3$ integrin opens, resulting in the high affinity form of the integrin head [31]. Thus, it is becoming increasingly evident that the hybrid domain in the β chain is critical for integrin activation, and a swing-out movement of this activates integrins (see Fig. 3).

Negative stain electron microscopy with image averaging of integrins has shown three overall conformations of the extracellular domain and these correspond to a low affinity, bent conformation (as in the $\alpha_V\beta_3$ structure), an intermediate affinity, extended form with a closed headpiece, and a high affinity, extended form with an open headpiece, which is induced by ligand mimetic compounds [32]. This model is also supported by crystal structures of integrins that lack the α I-domain, such as $\alpha IIb\beta_3$ [32]. In these integrins, the ligand binds directly to the β I-like domain, and causes a downward movement of its α_7 helix, similarly to what was described above for the integrin α I-domains. This shift results in a swing-out movement of the β chain hybrid and PSI domains and these domains act as a rigid lever that transmits and amplifies the motion, resulting in a separation of the α and β legs and integrin extension [32]. On the other hand, the swing-out movement of the upper β leg could readily occur if it were preceded by extension of both legs. In conclusion, transmembrane domain separation could occur as a later event during outside-in integrin activation, or as a key early step during inside-out activation [32].

Integrin conformation is dependent on the β chain, and an allosteric regulation of the α chain I-domain by the β chain I-like domain is essential. In particular, there is an invariant Glu (E310) in the linker formed by the α_7 helix and the β sheet 3 of the β_2 propeller domain, which is needed for I-domain activation [9,12,23,28]. This glutamate may act as a ligand for the β I-MIDAS, which drags the α_7 helix from its resting position, and thus activates the integrin α I-domain.

The importance of the close interaction between the α I-domain and the β I-like domain is underscored by the finding of small adhesion antagonist molecules, which bind to the β I-domains and inhibit the allosteric communication between I-domains [28]. Some monoclonal antibodies to the β_2 chain are also efficient blockers of adhesion and may act by influencing the α/β allosteric transitions [33,34].

2.2. The transmembrane domains

Less is known about the α/β transmembrane regions. The structure of $\alpha IIb\beta_3$ integrin β chain includes a 30 amino acid long transmembrane helix, which shows a tilt in lipid bilayers (bicelles) with a snorkeling of Lys-716 out from the lipid core followed by reinsertion in the membrane of the subsequent hydrophobic amino acids Leu-717–Ile-721 [35]. The helix tilt angle may specify the α/β transmembrane helix packing and control bidirectional signalling. In the presence of full-length β_3 cytoplasmic tail, helix propensity continues into the cytosol and may be stabilised by talin binding. In the α chain, the 29-residue transmembrane domain is formed by a 24-residue α-helix (Ile-966–Lys-989). Also in this case, the terminal residue is a lysine, which is snorkeling out of the membrane, followed by a reversed segment (Gly-991, Phe-992, Phe-993) that packs against the α-helix [36]. The Gly–Phe–Phe motif is fully conserved among human α integrins, and it may play a crucial role for the integrin transition from inactive to active: Phe/Ala double mutation leads to receptor activation [37], showing that these residues are important to keep the integrin in its resting state. Moreover, in the reported cytoplasmic $\alpha IIb\beta_3$ complex structure, which extends up to Lys-989, the two Phe residues are in helical conformation [38], suggesting that these residues might shift during integrins conformational changes.

In red cells glycophorin A displays an intramembrane GxxxG sequence, which is important in homodimerisation [39,40]. This conserved motif is also important in integrin α/β heterodimerisation, which happens preferentially when the interaction is studied on mammalian cell membranes [41]. Indeed, mutation of the two glycines in the GxxxG motif markedly reduces the α/β transmembrane interactions and fails to activate the integrin [41]. Moreover, there is a conserved valine in several integrin β chains (GVxxG) but this is replaced by a threonine in β_2 (T686). Importantly, when this was mutated to valine, LFA-1 was activated and cells adhered to intercellular adhesion molecule-1 (ICAM-1) [42]. The integrin showed relatively low affinity because it did not bind to ICAM-3, and it did not react with the intermediate affinity reporting antibody KIM127.

2.3. The cytoplasmic domains

With the exception of the β_4 integrin chain, the integrin cytoplasmic domains are generally relatively short and all are devoid of enzymatic activity (Fig. 4). Nevertheless, the cytoplasmic domains play a pivotal functional role in integrin activity. The α chain cytoplasmic domains show limited similarity, whereas the β chains are well conserved, suggesting similar properties. In particular, our knowledge of integrin cytoplasmic domains is based on the widely studied αIIbβ$_3$. Plow and coworkers have studied by NMR spectroscopy in water solution the cytoplasmic peptides including the regions at the transmembrane/cytoplasmic interphase. They found that proximal portions of the α and β cytoplasmic parts are α-helical and associate with each other [38,43]. The helices stretch from the membrane interphase to Arg-998 in αIIb and to His-722 in β_3 [44]. Importantly, the distal part of the β chain was ordered, and the proximal α-helix was followed by a NPXY loop and a short α-helix covering the residues from Tyr-747 to Thr-755. Very recent work shows that the cytoplasmic tail of the α_L integrin polypeptide forms a triple-helical structure; the α-helix 1 stretches from Lys-1093 to Met-1100, helix 2 from Ala-1112 to Glu-1123, and helix 3 from Lys-1131 to Gly-1142. Helix 3 makes contacts with both helix 1 and helix 2. Furthermore, helices 1 and 3 contact the β_2-tails in its N-terminal region, and the conformation of the β_2 chain changes after binding of activating talin [45]. Thus, there is vast data supporting the view that a close association of α and β chains in the cytoplasmic region occurs in integrins in the resting state, whereas chain separation results in activation of adhesion.

The GFFK(K)R sequence is well conserved in the α chains and is important in keeping the integrins in a non-adhesive state. When this motif is deleted or mutated, the integrins become activated [46,47]. It has been proposed that the arginine (995 in αIIb) in this sequence interacts with a juxtaposed aspartate (723 in β_3) in the β chains [37]. Kim et al. [41] showed that the integrin domains preferentially form heterodimers and the dimers are stabilised by the conserved cytoplasmic arginine-aspartic acid interaction. Importantly, talin was able to disrupt the association. Replacement of the α_L and β_2 cytoplasmic domains with salt bridge forming α-helical peptides inactivates LFA-1, whereas replacement with peptides which cannot dimerise causes activation. It is thought that this change in the cytoplasmic domains is then translated to further changes in the extracellular domains, resulting in integrin activation and ligand binding. However, mutation of the salt bridge *in vivo* did not yield a clear integrin phenotype, indicating that other events may occur [48]. Moreover, deletion of the GFFKR sequence in LFA-1 in mice, resulted in increased LFA-1 activation and LFA-1-dependent adhesion. However, the lack of LFA-1 deactivation resulted in impaired cell migration and inflammatory cell recruitment *in vivo* [49]. There are also other conserved sequences which are needed for integrin activity, for example the two β chain NPxY(F) sequences are functionally important [50] and are discussed below.

Fig. 4. The cytoplasmic sequences of the human α and β integrin chains (β_4 is not shown). The potential phosphorylation sites are marked in red. The established phosphorylation sites are numbered. The conserved membrane proximal sequences in the α chain are marked in brown. The functionally important NPXY(F) sequences in the β chain are marked in green and the threonine containing important phosphorylation sites in magenta.

3. Integrin ligands and inhibitors

Integrins bind to a large number of extracellular matrix molecules and cell membrane proteins. A full description of these is not possible here and falls out of the topic of this review, but the reader is referred to published review articles [4–6]. The $\alpha IIb\beta_3$ integrin binds to several ligands including fibrinogen, fibronectin and von Willebrand factor. Several β_1-family integrins bind to collagens, laminins, and fibronectin, but also to fibrinogen. It should be pointed out that some integrins show a high specificity for ligand binding, whereas others are more promiscuous and bind several different types of ligands.

A key observation was the identification of the RGD sequence in fibronectin and many other proteins, which is used as a common binding site for integrins [51], while several collagen binding integrins often recognise the characteristic tripeptide collagen repeats. The tripeptide RGD is a key lead structure in the development of anti-integrin competitive inhibitors [27,51,52]. Indeed, excess integrin activity can be deleterious, and therefore there is much interest in developing selective inhibitors of integrin activity. However, also excessive integrin inhibition can be deleterious: a natural example is provided by the snake toxins "disintegrins", which contain the RGD motif, and have devastating effects in humans [53].

Immunoglobulin superfamily members act as ligands for several integrins, and the best characterised integrin ligands are VCAM-1 (Vascular Cell Adhesion Molecule) and the ICAMs. VCAM-1 binds to $\alpha_4\beta_1$, $\alpha_V\beta_3$ and $\alpha_4\beta_7$, whereas the leukocyte-specific CD11/CD18 integrins (including LFA-1 and Mac-1) bind to ICAMs [54,55]. Five ICAMs are known: ICAM-1–ICAM-5 [6] (Fig. 5). ICAM-1 is expressed in many tissues including leukocytes and endothelial cells, and its expression is easily up-regulated upon cellular activation, for example by cytokines during inflammation [56]. ICAM-2 is found in leukocytes and endothelial cells [57,58], but it is more resistant to up-regulation [59]. However, it shows increased expression in malignant tissues [60]. ICAM-3 is expressed in leukocytes and is primarily important in immune responses [61,62]. ICAM-4 is red cell-specific [63], and recent work indicates that it may have a role in the removal of senescent cells by spleen macrophages [64]. ICAM-5 is solely expressed in brain neurons [65], and it shows both heterophilic and homophilic binding [66,67]. It strongly induces dendrite outgrowth [67], and upon glutamate receptor activation it is cleaved by the matrix metalloproteinases MMP-2/-9 [68]. Surprisingly, the soluble ICAM-5 fragment in turn causes inhibition of T lymphocyte activation, opposite to that of ICAM-1 [69]. This interesting molecule has recently been reviewed [70].

Leukocyte integrins are recognised therapeutic targets in various diseases, and integrin blocking monoclonal antibodies (natalizumab against α_4 integrins and efalizumab against LFA-1) are already used in the clinic against multiple sclerosis and psoriasis [71,72]. Also small molecule antagonists against leukocyte integrins are being developed. Interestingly, the statins, which are widely used for lowering cholesterol levels, have proved efficient inhibitors of leukocyte adhesion [73,74]. As an example, lovastatin binds at a crevice between the F-strand and the α I-domain of LFA-1 and inhibits allosteric movements [9]. Several monoclonal adhesion blocking antibodies bind to the β chains and they may act by inhibiting the allosteric movements needed for integrin activation [75]. Because phosphorylation of integrin cytoplasmic tails show functional effects it would be important to develop drugs that specifically interfere with the integrin phosphorylations. Such drugs could affect cell adhesion, movement or other integrin-dependent functions [76]. However, little has yet been done in this field.

Leukocyte adhesion cannot be inhibited by compounds containing the RGD sequence, but longer peptides to recognition sites in ICAM-molecules or microbe-derived ICAM-1 inhibitors [77,78] do show inhibitory activity [79,80]. Soluble ICAM-1 and -2 are found in plasma and they show inhibition of adhesion, but are not very efficient, due to low affinity for the integrins. A promising approach could be to disrupt the association between the matrix metalloproteases and integrins [15,16].

Recently, it was found that the Del-1 protein is an important endogenous inhibitor of leukocyte adhesion [81]. The Del-1 protein is a secreted protein expressed by endothelial cells in immunoprivileged tissues, such as the brain, the eye, and the lung. Although a secreted molecule, Del-1 is absent from plasma and is rather localised to endothelial cells and/or the extracellular matrix [81,82]. In fact, it binds to the β_2 integrins (LFA-1, Mac-1) and when coated on plastic, leukocytes adhere to the protein. However, leukocyte binding to ICAM-1 is inhibited when both Del-1 and ICAM-1 are present. Del-1 knockout mice show a strong activation of adhesion and of inflammatory cell recruitment [81].

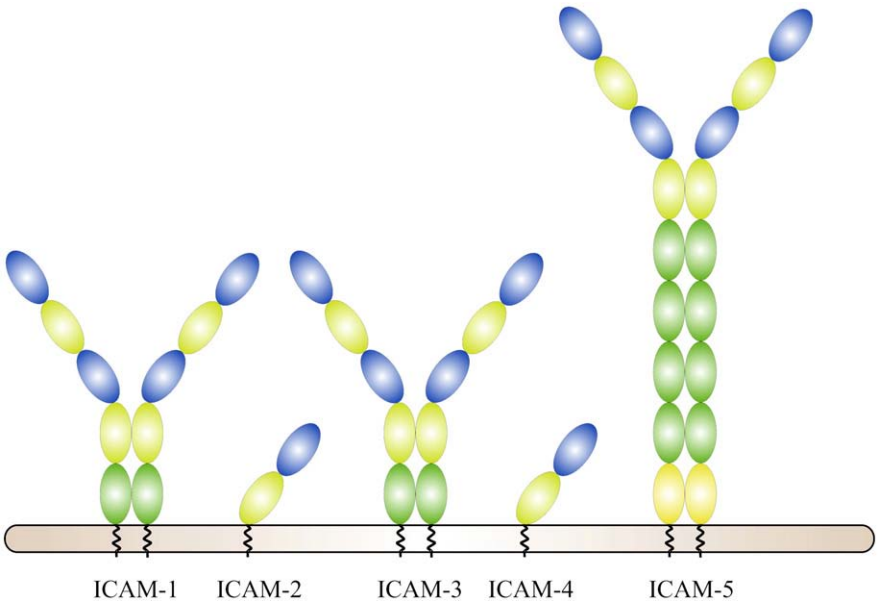

Fig. 5. Schematic structures of ICAMs. Similar Ig-like domains are colour coded. ICAM-1 and ICAM-3 are dimers, ICAM-2 and ICAM-4 monomers. ICAM-5 may exist as a dimer or tetramer.

4. Conformational versus valency regulation of integrins

Because integrins perform a number of different functions including adhesion and signalling in two directions we could anticipate that several types of mechanisms must exist to achieve these goals. Furthermore, it is obvious that integrins in some cases must be able to react rapidly, whereas in other instances this is not that important. An example of the former is the adhesion of leukocytes to endothelial cells in blood vessels [83,84], whereas an example of a slower reaction could be the formation of the immunological synapse between a T cell and an antigen-presenting cell during an immune response and subsequent signalling [85].

As discussed in the section on integrin structure, there is ample proof that conformational changes occur in integrins. These involve changes in integrin-binding *affinity*. Another mechanism for integrin activation includes clustering, so that an *increased valency* results in increased ligand binding through higher *avidity*. Obviously, combinations of these two major mechanisms are possible (Fig. 3).

4.1. Outside-in activation involves changes in integrin conformation — allosteric regulation

Several experiments show that ligand binding to the external domains of integrins induce conformational changes, which may increase ligand affinity. Subsequently, signals may be generated through alterations in the cytoplasmic domain structures.

Most molecular work done on outside-in integrin activation deals with the leukocyte and platelet integrins. In particular, β_2 integrins constitute excellent models for research on integrin regulation. There are several reasons for this: leukocytes are easily obtained, the cells grow in suspension, a number of cell lines are available and for example T lymphocytes exist in a truly resting state, but can easily be activated by both outside-in and inside-out activations. Furthermore, a vast amount of structural and functional information is available on β_2 integrins, notably LFA-1 and Mac-1. In these integrins, the I-domain, which forms the ligand binding site, is of pivotal importance. The binding of ICAM-5 to the LFA-1 I-domain has recently been studied in atomic detail. Upon binding a remarkable outward movement of the α_7 helix was observed [86]. This resulted in the replacement of the corresponding α_7 helix from a neighbouring I-domain into the α_7 helix position, but in an upside-down configuration. This α_7 helix replacement was further propagated, resulting in a large I-domain/ICAM-5 cluster. In this way, a weak initial interaction between the integrin and a ligand can result in the formation of large ligand/receptor aggregates. Whether this occurs with the whole integrin and in the cell membrane is not known, but integrin clustering (valency increase) is certainly a major mechanism of adhesion. The $\alpha_2\beta_1$ integrin also contains an I-domain and the binding to a collagen triple-helical peptide has been studied at the atomic level [87], showing similar mechanisms.

It is becoming increasingly apparent that the integrin β polypeptides have an important role in integrin activity regulation, and also in ligand binding. As discussed above, the β I-like domain regulates allosterically the α I-domain ligand affinity. In particular, the ADMIDAS site interaction with the MIDAS site does not occur upon activation, allowing remodeling of the ligand binding site [9,12]. Stabilisation of the α_7 helix structure impairs integrin affinity regulation and leads to a LAD-I phenotype. This was obtained by making the mutation N329S in the β I-like domain [88].

The outside-in activation propagates signals to the cytoplasm. In elegant experiments Springer and coworkers have studied the signalling by using chimeric α_L-cyan fluorescent proteins and β_2-yellow fluorescent proteins [89]. When transfected into cells the cytoplasmic fluorescent proteins were closely associated giving positive FRET signals. Upon outside-in activation using Mn^{+}, ICAM-1, or an LFA-1 activating antibody, the FRET signals disappeared, evidently due to increased distances between the cytoplasmic integrin domains.

4.2. Interactions between integrin cytoplasmic domains and intracellular factors regulate integrin activity and ligand binding: inside-out activation

From where do the structural changes explained above originate? In most cases of integrin activation, signals originate from various cell surface receptors, which propagate them into the cell. Intracellular signalling pathways leading from receptors to integrin activation have been discussed in several recent reviews [7–12,90], and the reader is referred to these for further information. For the β_2 integrins such as LFA-1, these events have been extensively studied in T cells, where integrin inside-out activation can be initiated by ligation of the T cell receptor or chemokine receptors.

More proximal to the integrin receptor, it is thought that structural changes/valency changes in the integrin are mediated by the regulated interaction of the integrin cytoplasmic domains with intracellular factors. Indeed, a large number of proteins have been described to directly interact with integrin cytoplasmic domains, including cytoskeletal proteins (talin, filamin, alpha-actinin, kindlins), small G proteins and GEFs (cytohesin), adaptor proteins (14-3-3), kinases (protein kinase D), and even transcriptional coactivators (JAB-1). At least some of these proteins have overlapping binding sites in the integrin cytoplasmic domains; thus, spatiotemporal regulation of these interactions must be important. Below are described some of the factors binding to integrin cytoplasmic tails.

4.3. Important cytoplasmic integrin regulators

Talin is a 270 kDa cytoplasmic protein with a globular head and a flexible rod region [91]. Importantly, the head region of talin can activate integrins. The head contains a FERM domain (Protein 4.1, Ezrin, Radixin, Moesin) which has F1, F2 and F3 subdomains. The interaction of the F3 domain with the β_3 integrin has been extensively studied, and the structure of the F3 domain with an integrin cytoplasmic peptide has been determined [92,93]. The head domain binds to the proximal NPXY (F) in integrin β chains, with the tyrosine inserting into a hydrophobic product in F3, as does Trp-739 from the β_3 integrin. The integrin peptide adopts a β-strand conformation followed by a reverse turn [92]. Additionally, the F3 domain interacts hydrophobically with the membrane proximal α-helical region of the integrin cytoplasmic domain [93,94], and is thus positioned to affect the activation state of the integrin by perhaps affecting the association between the α and β integrin polypeptides. Importantly, disruption of the talin gene in platelets leads to impaired integrin activation, demonstrating the crucial role of this protein in integrin regulation [95].

It has now been shown that the talin head domain has a Kd of 0.1–0.5 μM for the β_2-tail [45,96] whereas the Kd for the affinity of α_L to β_2 is 2.6 μM [45]. This finding explains the fact that talin is able to disrupt the α_L and β_2 association. It is thought that talin is recruited to the integrin tails by the Rap1-GTP-interacting-adaptor molecule (RIAM). Indeed, RIAM overexpression stimulates $\alpha IIb\beta_3$, and induces adhesion, whereas a knock-down blocks it [97]. The G protein exchange factor CalDAG-GEF1 is also needed for Rap1 activation, and in this way it participates in the Rap1-talin activation of integrins [98]. For example, in the rare LAD-I variant (LAD-III) syndrome CalDAG-GEF1 is often mutated and integrin signalling is impaired [99].

The kindlin family of proteins consists of three members, kindlin-1,-2 and -3 [100]. Kindlin-1 is mutated in the Kindler syndrome, where skin blistering occurs due to failure of actin function in keratinocytes. Kindlin-2 is widely expressed and interacts with integrin-linked-kinase (ILK) and migfilin [101]. Kindlin-3 is confined to hematopoietic cells [102] and may regulate cell apoptosis by acting as a transcriptional repressor in NF-kB signalling [103]. Interestingly, patients who

were diagnosed with the LAD-I variant syndrome have mutations in their kindlin-3 gene and in most cases [104], but not always, in their CalDAG-GEF1 gene [105]. This shows that the syndrome is due to defective kindlin-3 [104]. Kindlin-2 is known to bind to the T-759ST-NITY region of the β_3 integrin polypeptide and acts synergistically with talin in integrin activation [106] and the binding site for kindlin-3 is in the same region [102].

Similarly to talin, α-actinin can also bind both actin and integrin β chains. The binding site is located in the membrane proximal region of the β_2 integrin polypeptide, while the membrane distal portion has an inhibitory effect on these interactions [107] (Fig. 6). Recently, it has been shown how α-actinin links LFA-1 to the cytoskeleton, and disruption of such binding results in loss of cell spreading and migratory speed. Furthermore, α-actinin co-localised and could be immune precipitated with KIM127 positive LFA-1 molecules, which shows that intermediate affinity integrins and α-actinin form a complex [108].

Filamin is a large cytoskeletal molecule that has also been shown to bind to integrin β chains [109]. Its binding site in the integrin is partially overlapping with talin (Fig 6) and indeed, filamin appears to be a negative regulator of at least β_7 and β_2 integrin ligand binding and of cell migration [110]. The crystal structure of the integrin-binding Ig-like domain (domain 21) of filamin in complex with β_7 and β_2 integrin peptides has been solved [96,111], and shows that the integrin peptide forms extended β-strands that interact with strands C and D in the Ig-like domain-21 of filamin (Fig. 7).

The cytohesins 1–4 are nucleotide exchange factors (GEF) for the ARF family of small G proteins [112]. Cytohesin-1 binds through its central domain to the proximal part of the β_2-subunit, whereas its pleckstrin homology domain interacts with the plasma membrane lipids. The β_2-interaction results in up-regulation of LFA-1 activity. GEF activity is not needed in this context [113], but it is required for cell spreading. Cytohesin-3 has a similar activity as cytohesin-1 [114], whereas cytohesin-2 regulates cell motility [115,116]. Cytohesin-1 is also implicated in Mac-1 inside-out signalling through the CD14/Toll-like receptor in monocytes [117].

The 14-3-3 proteins are small dimeric adapter proteins that bind to Ser/Thr phosphorylated sequences in proteins and alter protein localisation or activity. Phosphorylation of β_2 polypeptides on Thr-758 results in recruitment of 14-3-3 proteins [118]. 14-3-3 only binds to the phosphorylated peptide in the well characterised binding site between 14-3-3 α-helices E and F [96]. This interaction is functionally important, because when it is inhibited by a T/A mutation in the integrin, adhesion to ICAM-1 is reduced [118]. The inhibition can also be achieved by using a construct that blocks the phosphopeptide binding site in 14-3-3. Inhibition of the 14-3-3/integrin-binding efficiently impairs cell spreading on ICAM-1. The Cbl-b protein is an adaptor protein and a ubiquitin ligase, which has been shown to affect leukocyte adhesion. T cells deficient in Cbl-b showed enhanced adhesion to ICAM-1 [119]. Importantly, Cbl-b deficiency results in increased phosphorylation of T758, followed by enhanced binding of 14-3-3 proteins and increased LFA-1 activity [120]. Interestingly, the threonines 758–760 in β_2 are also essential for the accumulation of

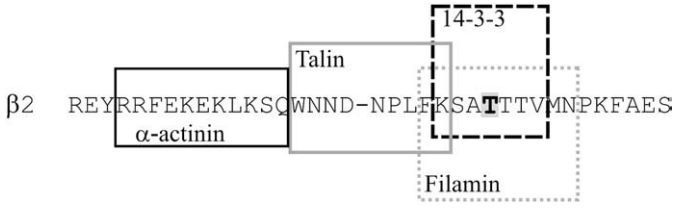

Fig. 6. Overlapping binding sites for cytoskeletal proteins in the β_2 cytoplasmic segment. The functionally important threonine-758 residue is shown in bold. Phosphorylation of this residue enables binding of 14-3-3 proteins but inhibits filamin binding.

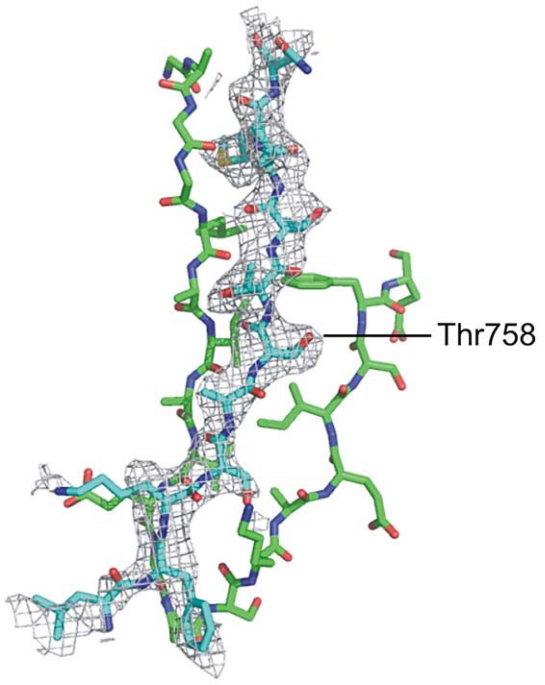

Fig. 7. The crystal structure of the filamin domain 21 (green) binding region in complex with the β_2 peptide. The important Thr-758 is shown. When this becomes phosphorylated hydrophobic interactions are disturbed and there is no space for the peptide in the filamin binding site and binding becomes impossible.

the small G protein Rho in its active GTP form at Mac-1 containing phagosomes, showing that also other β_2 integrins may be regulated by this phosphorylation event [121].

Importantly, these structural studies have shown that the integrin cytoplasmic tails may adopt different conformations depending on which cytoplasmic partner is bound to it. Additionally, the kindlin studies have revealed that also other proteins than talin may play important roles in integrin activation *in vivo*.

Integrin inside-out and outside-in activations are regulated by phosphorylations. Integrin directional signalling is regulated by cytoplasmic proteins. How are these molecular interactions with integrin cytoplasmic domains then regulated? At least *competition* and *phosphorylation* appear to regulate binding of cytoplasmic molecules to the integrin tails. This is an area of research which is still relatively underexplored, but details of which are currently emerging. The most common way to regulate protein (enzyme) activities is by phosphorylation and dephosphorylation of serine/threonine and tyrosine residues. A characteristic feature of this modification is the possibility of rapid reactions, especially when compared to the slower changes regulated at the transcription and translation levels. Although integrin phosphorylation has been studied for more than 20 years, major developments occurred only recently [76].

4.4. β_2 integrin chain phosphorylation

The cytoplasmic sequences of the α and β chains of LFA-1, Mac-1, $\alpha_x\beta_2$ and $\alpha_D\beta_2$ are shown in Fig. 4 with the potential phosphorylation sites marked in red. From early on it was noted that α chains are constitutively phosphorylated, whereas the β chain is not [122–125]. Both phorbol esters and T cell receptor antibodies could induce phosphorylation of threonine residues in the β tail [[126–129]. These threonines are important for adhesion to ICAM-1 [125]. In addition, Ser-745 is phosphorylated and several isoforms of protein kinase C can phosphorylate the β_2 chain cytoplasmic peptide *in vitro* [128]. Ser-756 is strongly phosphorylated when T cells are activated with phorbol

esters [125], but not by activation of the T cell receptor [129]. This could mean that the serine phosphorylation is an experimental artifact or it is involved in T cell functions not related to antigen activation. Tyr-735 is phosphorylated in interleukin-2 treated natural killer cells [130], and also in neutrophils after binding to collagen [131]. Whether this phosphorylation results in recruitment of cytosolic proteins is not known. A schematic view of the T cell receptor initiated phosphorylation of the β_2 chain is shown in Fig. 8.

4.5. α_L and α_M integrin polypeptide phosphorylations

The phosphorylation site in α_L turned out to be Ser-1140 [118], and interestingly cellular activation through chemokines, or outside-in activation through soluble ICAM-2 or Mg^{+} treatment was attenuated in S1140A mutated cells. Furthermore, the α_L non-phosphorylated variant can form an integrin heterodimer, but the mutation affected its ability to induce conformational changes in the integrin. Thus the S1140A mutation resulted in inability to bind soluble ligand (ICAM-1), and the activation epitope for the monoclonal antibody Mab24 was not induced by the activating antibody MEM83 [118]. However, use of the KIM127 antibody has shown that the ability to form an extended form is not lost by the mutation (unpublished). These results indicate that rearrangements within the α_L I-domain need phosphorylation of Ser-1140 to become fully active.

Further work showed that α_M phosphorylation takes place on Ser-1126. Mutation of this residue resulted in impairment of transfected cells to leave the blood. Whereas wild-type cells largely accumulated in the lungs and spleen of mice injected for a short time with human cells, Ser-1126 mutated cells remained in the circulation [132]. In contrast to the situation with LFA-1, Mac-1 extension upon activation did not occur in S1126 mutated cells as detected with KIM127. This result shows that the β_2 integrins are differently regulated by cytoplasmic phosphorylations.

4.6. Phosphorylation of β_1 and β_3 integrins

As compared to the extensive work on various aspects of β_2 and β_3 integrins, there are relatively few studies on the phosphorylations of β_1 integrins. The β chains contain the two functionally important cytoplasmic NPxY/F sequences (Fig. 4). In β_1 they are located at residues N-780PIY and N-792PKY and in β_3 at N-744PLY and N-756ITY. The β_1 tyrosine residues may be phosphorylated, but phosphorylations do not seem important in this case, because mutations to phenylalanines have no effect. However, mutations to alanines resulted in β_1-null phenotypes *in vivo* [48].

Thr-788, which corresponds to the first threonine in the β_2-threonine triplet is important for β_1 integrin function [133]. Mutation to alanine reduced cell attachment to fibronectin, whereas the phosphorylation-mimicking mutation T788D was similar to wild-type integrins. However, it induced an increased number of focal contacts and the cells migrated more slowly.

The $\alpha_4\beta_1$ integrin is important for leukocyte migration and inflammation. Ser-988 in the α chain is phosphorylated, possibly by protein kinase A [134,135]. Mutation of this residue to alanine reduced

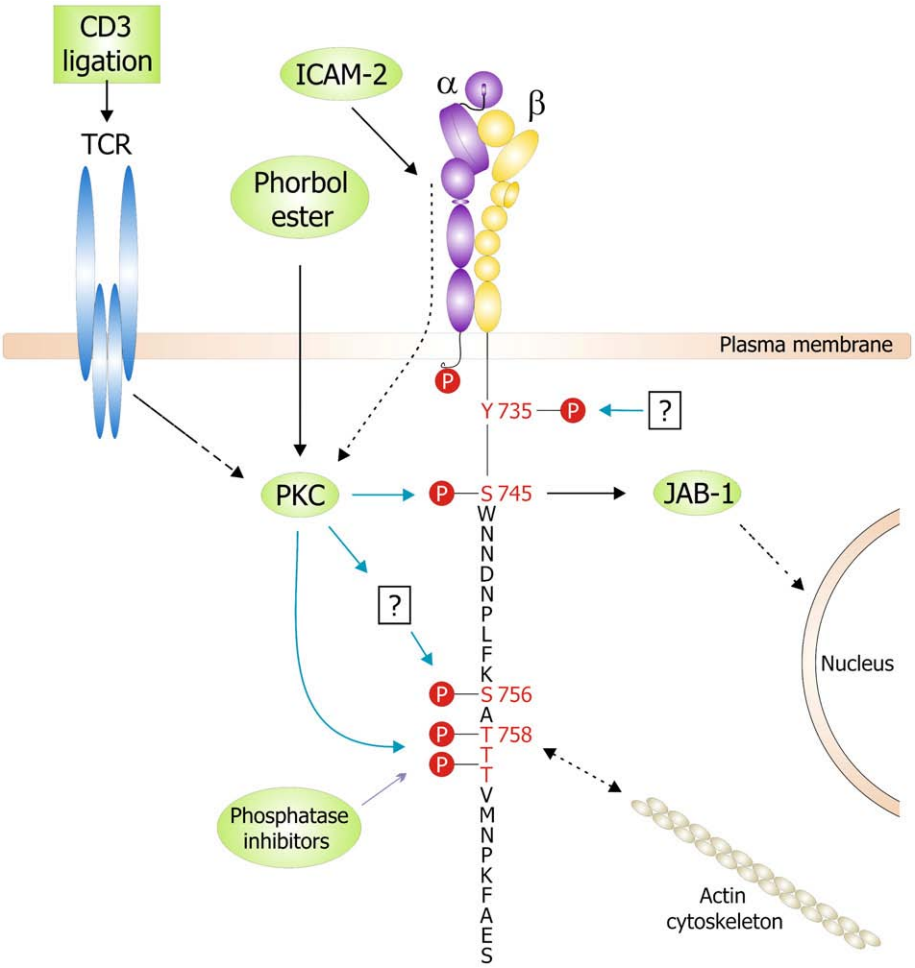

Fig. 8. Phosphorylation of the β_2 chain. Phosphorylation of β_2 through the T cell receptor results in downstream events affecting integrin activation through avidity and affinity modulations.

cell spreading and migration. On the other hand, a S988D mutation inhibited cell migration and promoted cell spreading. This could mean that both phosphorylation and dephosphorylation at Ser-988 are functionally important. The phosphorylated form of $\alpha_4\beta_1$ preferentially located to the leading edge of cells where active protein kinase A is present, indicating that localised activation of $\alpha_4\beta_1$ takes place through a local activation of the protein kinase.

Outside-in binding of $\alpha IIb\beta_3$ ligands resulted in further activation and tyrosine phosphorylation of the tyrosines in the two β_3 NPxY sequences [136]. These phosphorylations induced signalling and platelet aggregation. The tyrosines are needed for cell spreading and transfer of the integrins to focal adhesion sites [50]. The β_3 phosphorylated Tyr-759 made the β_3 chain resistant to calpain cleavage, whereas the dephosphorylated chain could be cleaved, and this inactivated integrin signalling and cell spreading [137]. The β_3 polypeptide in $\alpha_V\beta_3$ is evidently phosphorylated when bound to vitronectin but not to fibronectin [138,139], which shows that the ligand may affect tyrosine phosphorylation.

4.7. Phosphorylation of integrin tails affects cytoskeletal interactions and signalling

In the previous chapter we have described phosphorylation in the cytoplasmic tails of integrins. But how do the phosphorylations mediate further cellular effects? Recent work has partially elucidated the mechanisms. It is evident that phosphorylation enables integrins to regulate their interactions with adaptor and cytoskeletal proteins, resulting in subsequent downstream effects. A number of cytoplasmic proteins affect integrin-dependent signalling and adhesion, and our current picture of these events is still a simplified version which is far from complete.

Thr-758 phosphorylation directly regulates the binding of 14-3-3 proteins and filamin to the β_2 integrin cytoplasmic domain. 14-3-3 only bound to the phosphorylated form, while filamin only bound to the non-phosphorylated form of the integrin [96] (Fig. 6). X-ray crystallography experiments clearly showed how phosphorylation of the tail works as a molecular switch to change the binding [96] (Fig. 7). When Thr-758 was phosphorylated, filamin binding was inhibited because the hydrophobic interaction between filamin and Thr-758 in the integrin was disrupted. Talin binding was not directly affected by Thr-758 phosphorylation of LFA-1, but 14-3-3 and talin binding sites in the integrin are partially overlapping, and 14-3-3 binding out-competed the binding of talin to the integrin cytoplasmic tail [96].

Thus both phosphorylation and competition between these proteins regulate interactions with the integrin cytoplasmic domains. Additional complexity is added by other cytoplasmic proteins. Recent work shows that the adaptor protein migfilin binds to filamins-1, -2 and -3 in a very similar mode as the β_2 and β_7 integrin tails [140]. It dissociated filamin from integrin β-tails, and promoted talin binding and integrin activation [141].

A conceptually similar switch of integrin activation as for β_2-filamin/talin/14-3-3 was recently reported for the β_3 integrin chain [142]. Talin bound well to the β_3 cytoplasmic peptide, but upon phosphorylation of Tyr-747 in the proximal canonical NPXY motif in

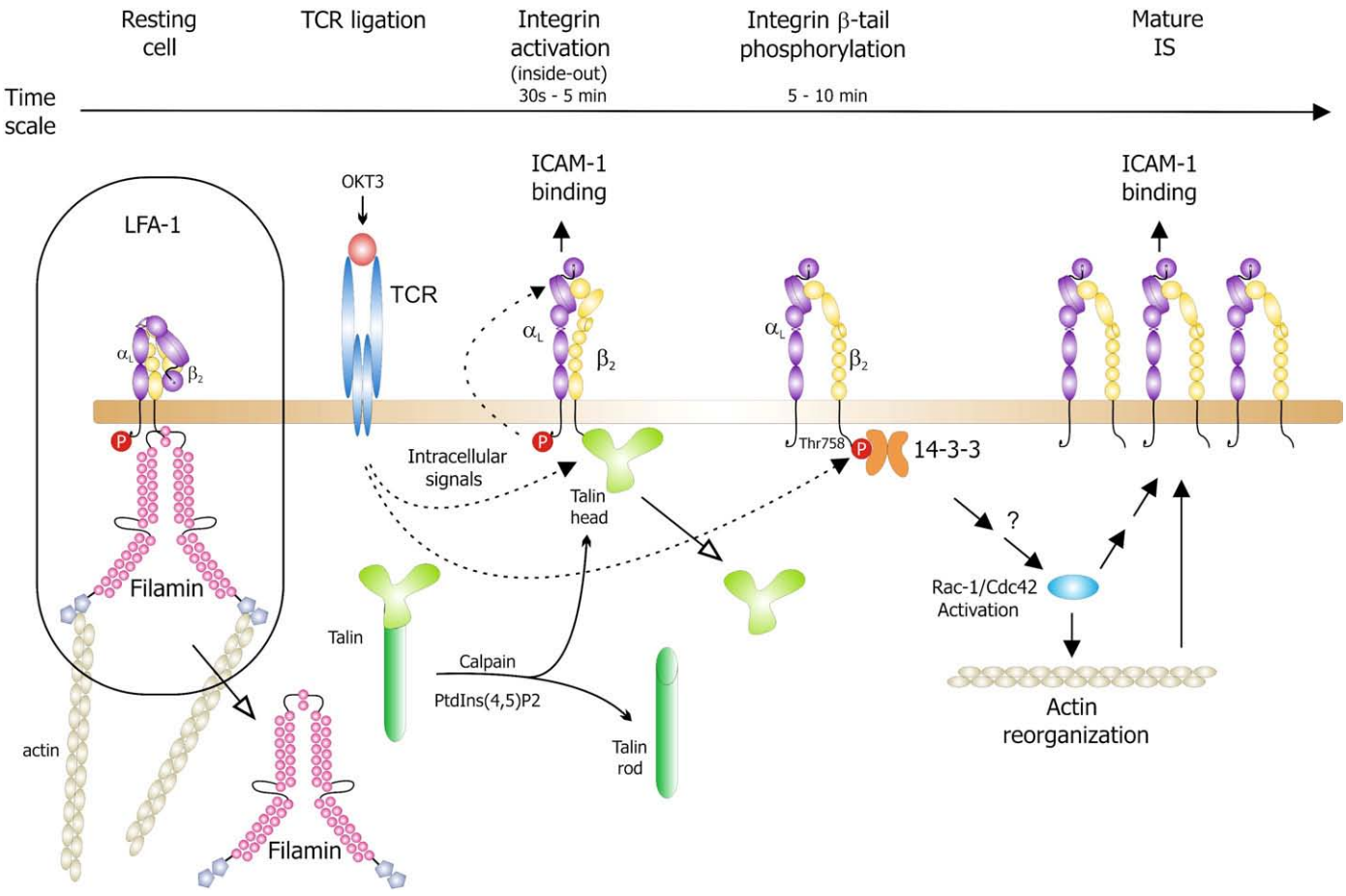

Fig. 9. A schematic and partially hypothetical view of LFA-1 activation. In the resting state (left) filamin is bound to the integrin and the ligand binding site is closed. Upon activation through the T cell receptor, talin may be cleaved by activated calpain, which results in binding of the talin head to the integrin cytoplasmic tails and separation of the integrin chains. Then phosphorylation of β_2 (Thr-758) results in 14-3-3 binding, displacement of talin and activation of Rac1/Cdc42, which affect the actin cytoskeleton resulting in integrin clustering. The integrin is here shown in extended forms.

the integrin, it was replaced by the cytoplasmic protein Dok1. Also here phosphorylation of the integrin regulates the binding of cytoplasmic factors to the integrin tail, demonstrating that this may be a universal principle in the regulation of integrin signalling.

The phosphorylation of the β_2 integrin polypeptide at Thr-758 further activates integrin inside-out signalling. This was seen by subsequent activation of the small G proteins Rac1 and Cdc42 [143]. Activation of Rac1 is known to induce the formation of actin polymers resulting in lamellipodia and membrane ruffles [144]. Activation of Cdc42 in turn results in the formation of filopodia [144]. 14-3-3 proteins do not bind to these G proteins directly, and therefore there must exist adaptor molecules between 14-3-3 and the G proteins. One possible candidate is Vav-1. Upon activation of integrin signalling, Vav-1 was phosphorylated and cell spreading was induced [145]. Consistently, Vav-1/Vav-3-deficient neutrophils displayed impaired β_2 integrin-dependent adhesion and spreading [146]. Another candidate is the Rac1 nucleotide exchange factor Tiam1. Tiam1 can activate Rac1, but not Cdc42 [147,148] and it is involved in T cell trafficking [149]. The Cdc42 protein can induce cell polarity after β_1 integrin activation, because in migrating astrocytes an RGD peptide completely inhibited Cdc42. Furthermore, Cdc42 recruited a partitioning defective polarity complex (Par) containing PKC([150]. A schematic view of these events is shown in Fig. 9.

The functional significance of the other phosphorylation sites in β_2 is not well understood. Ser-745 is phosphorylated in β_2 [128], and this phosphorylation is induced after treatment of cells with LFA-1 antibodies or the ligand ICAM-2 [151]. The phosphorylation resulted in disengagement of the transcriptional activator JAB-1 from LFA-1, and this triggered further downstream signalling events. This would thus be an example of outside-in signalling where phosphorylation is important.

The α_L phosphorylation on Ser-1140 and the Ser-1126 phosphorylation of α_M are functionally important, but little is known about which kinases are involved or how the phosphorylations affect function. The integrin α chain phosphorylations affect integrin conformations as shown by monoclonal reporter antibodies and increased affinity of integrins for ligands. A constitutively active Rap1 G protein has been shown to activate T cells and to induce adhesion to ICAM-1 [152,153]. Interestingly, it could not activate the α_L phosphorylation site mutant [118]. Rap1 in turn binds to the RAPL protein [154]. RAPL was found to regulate the Mst1 protein kinase and they formed a complex at the leading edge of cells [155]. Mst1 is needed for the downstream effects of RAPL. Rap1 requires Cdc42 activity and Tiam1 has been found to associate with Rap1 and the Par complex [156]. In migrating T cells, Tiam1 and the Par complex are required to induce polarity with LFA-1 at the leading edge. Thus there may exist a crosstalk between the α_L and β_2 phosphorylations through these G proteins and nucleotide exchange factor proteins.

Paxillin is an adaptor protein, which binds to the α_4 chain resulting in increased cellular migration but reduced spreading [134,157]. The Ser-988 phosphorylation inhibited paxillin binding. When the residue was mutated to aspartic acid, which at least partially mimics phosphorylation, the paxillin binding was inhibited and the cells showed effects similar to that of the phosphorylated integrin. When mutated to alanine, cell migration was reduced, due to paxillin binding. These results indicate that phosphorylation/dephosphorylation at Ser-988 is needed for physiological cell migration.

4.8. Different protein phosphatases are important in integrin regulation

The phosphatase 2A dephosphorylates Ser/Thr residues and is blocked by okadaic acid and calyculin A. It can bind to the proximal cytoplasmic sequence KVGFFKR in αIIb and interestingly it blocks $\alpha IIb\beta_3$ signalling [158]. Binding of collagen to the $\alpha_2\beta_1$ integrin activated the 2A phosphatase, which resulted in dephosphorylation of the Akt and glycogen synthase kinase 3β [159]. On the other hand, activation of the tyrosine phosphatase TCPTP by the $\alpha_1\beta_1$ integrin, down-regulated epidermal growth factor receptor signalling [160]. These results further support the view that not only kinases, but also phosphatases are involved in regulation of integrin activity.

The fact that inside-out signalling results in activation of integrin-binding to ligands, and initially weak ligand binding from the outside can result in stronger binding and adhesion (see ICAM-5 [86]), shows that these two events are intimately connected. This would mean that in many instances a functioning adhesion complex is built up from integrin ligand interactions with integrins up-regulated both by changes in affinity and avidity.

In order to understand complex biological phenomena, scientists have often turned to simplified experimental setups. This certainly also holds true in integrin research: much work has been done with purified integrins, or I-domains and their ligands. This type of research has been very rewarding and yielded to a vast amount of useful data. However, it is becoming increasingly apparent that cellular adhesion is unusually complex, and in order to be able to make meaningful conclusions we have to study even more in molecular detail the events occurring at the cellular and organism levels. It is also important to include more temporal aspects in adhesion research, especially when various intracellular proteins compete for integrin-binding during activation. Many of them act indirectly through regulation of integrin-mediated adhesion, and therefore the adhesion field is becoming even more challenging to understand. The use of partially reconstituted systems may turn out necessary, but we have to develop new methods and increasingly turn to animal models. This will be a long, but interesting, scientific journey.

Acknowledgements

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References

[1] M. Takeichi, Cadherin cell adhesion receptors as a morphogenetic regulator, Science 251 (1991) 1451–1455.
[2] R. González-Amaro, F. Sanchez-Madrid, Cell adhesion molecules: selectins and integrins, Crit. Rev. Immunol. 19 (1999) 389–429.
[3] S. Yona, H.-H. Lin, W.O. Siu, S. Gordon, M. Stacey, Adhesion-GPCRs: emerging roles for novel receptors, Trends Biochem. Sci. 33 (2008) 491–500.
[4] R.O. Hynes, K.M. Yamada, Fibronectins: multifunctional molecular glycoproteins, J. Cell Biol. 95 (1982) 369–377.
[5] B.A. Cunningham, J.J. Hemperly, B.A. Murray, E.A. Prediger, R. Brackenbury, G.M. Edelman, Neural cell adhesion molecule: structure, immunoglobulin-like domains, cell surface modulation, and alternative RNA splicing, Science 236 (1987) 799–806.
[6] C.G. Gahmberg, Leukocyte adhesion. CD11/CD18 integrins and intercellular adhesion molecules, Curr. Opin. Cell Biol. 9 (1997) 643–650.
[7] F.G. Giancotti, E. Ruoslahti, Integrin signaling, Science 285 (1999) 1028–1032.
[8] R.O. Hynes, Integrins: bidirectional, allosteric signaling machines, Cell 110 (2002) 673–687.
[9] M.A. Arnaout, B. Mahalingam, J.-P. Xiong, Integrin structure, allostery, and bidirectional signaling, Annu. Rev. Cell Dev. Biol. 21 (2005) 381–410.
[10] M.H. Ginsberg, A. Partridge, S.J. Shattil, Integrin regulation, Curr. Opin. Cell Biol. 17 (2005) 509–516.
[11] T. Kinashi, Intracellular signalling controlling integrin activation in lymphocytes, Nat. Rev. Immunol. 5 (2005) 546–559.
[12] B.-H. Luo, C.V. Carman, T.A. Springer, Structural basis of integrin regulation and signaling, Annu. Rev. Immunol. 25 (2007) 619–647.
[13] D.C. Anderson, T.A. Springer, Leukocyte adhesion deficiency — an inherited defect in the Mac-1, LFA-1 and P150,95 glycoproteins, Annu. Rev. Med. 38 (1987) 175–194.
[14] M.A. Arnaout, Leukocyte adhesion molecules deficiency: its structural basis, pathophysiology and implications for modulating the inflammatory response, Immunol. Rev. 114 (1990) 145–180.
[15] M. Stefanidakis, M. Björklund, E. Ihanus, C.G. Gahmberg, E. Koivunen, Identification of a negatively charged peptide motif within the catalytic domain of progelatinases that mediates binding to leukocyte β_2 integrins, J. Biol. Chem. 278 (2003) 34674–34684.

[16] M. Stefanidakis, T. Ruohtula, N. Borregaard, C.G. Gahmberg, E. Koivunen, Intracellular and cell surface localization of a complex between $\alpha_M\beta_2$ integrin and promatrix metalloproteinase-9 progelatinase in neutrophils, J. Immunol. 172 (2004) 7060–7068.
[17] J. Zhu, C.V. Carman, M. Kim, M. Shimaoka, T.A. Springer, B.-H. Luo, Requirement of α and β subunit transmembrane helix separation for integrin outside-in signaling, Blood 110 (2007) 2475–2483.
[18] D.M. Rose, R. Alon, M.H. Ginsberg, Integrin modulation and signaling in leukocyte adhesion and migration, Immunol. Rev. 218 (2007) 126–134.
[19] B.H. Luo, C.V. Carman, T.A. Springer, Structural basis of integrin regulation and signaling, Annu. Rev. Immunol. 25 (2007) 619–647.
[20] R.O. Hynes, Integrins: a family of cell surface receptors, Cell 48 (1987) 549–554.
[21] J.-P. Xiong, T. Stehle, B. Diefenbach, R. Zhang, R. Dunker, D.L. Scott, A. Joachimiak, S.L. Goodman, M.A. Arnaout, Crystal structure of the extracellular segment of integrin αvβ3, Science 294 (2001) 339–345.
[22] T.A. Springer, Folding of the N-terminal, ligand-binding region of integrin α-subunits into a β-propeller domain, Proc. Natl. Acad. Sci. U. S. A. 94 (1997) 65–72.
[23] J.-O. Lee, P. Rieu, M.A. Arnaout, R. Liddington, Crystal structure of the A domain from the α subunit of integrin CR3 (CD11b/CD18), Cell 80 (1995) 631–638.
[24] A. Qu, D.J. Leahy, The role of the divalent cation in the structure of the I domain from the CD11a/CD18 integrin, Structure 4 (1996) 931–942.
[25] J. Zhu, B.-H. Luo, T. Xiao, C. Zhang, N. Nishida, T.A. Springer, Structure of a complete integrin ectodomain in a physiologic resting state and activation and deactivation by applied forces, Mol. Cell 32 (2008) 849–861.
[26] J.-P. Xiong, T. Stehle, R. Zhang, A. Joachimiak, M. Frech, S.L. Goodman, M.A. Arnaout, Crystal structure of the extracellular segment of integrin αVβ3 in complex with an Arg-Gly-Asp ligand, Science 296 (2002) 151–155.
[27] T.A. Springer, J. Zhu, T. Xiao, Structural basis for distinctive recognition of fibrinogen (C peptide by the platelet integrin $\alpha_{IIb}\beta_3$, J. Cell Biol. 182 (2008) 791–800.
[28] N. Nishida, C. Xie, M. Shimaoka, Y. Cheng, T. Walz, T.A. Springer, Activation of leukocyte β_2 integrins by conversion from bent to extended conformations, Immunity 25 (2006) 583–594.
[29] J. Takagi, B.M. Petre, T. Walz, T.A. Springer, Global conformational rearrangements in integrin extracellular domains in outside-in and inside-out signaling, Cell 110 (2002) 599–611.
[30] M.K. Robinson, D. Andrew, H. Rosen, D. Brown, S. Ortlepp, P. Stephens, E.C. Butcher, Antibody against the Leu-CAM β-chain (CD18) promotes both LFA-1- and CD3-dependent adhesion events, J. Immunol. 148 (1992) 1080–1085.
[31] E. Puklin-Faucher, M. Gao, K. Schulten, V. Vogel, How the headpiece hinge angle is opened: new insights into the dynamics of integrin activation, J. Cell Biol. 175 (2006) 349–360.
[32] T. Xiao, J. Takagi, B.S. Coller, J.-H. Wang, T.A. Springer, Structural basis for allostery in integrins and binding to fibrinogen-mimetic therapeutics, Nature 432 (2004) 59–67.
[33] F. Sánchez-Madrid, P. Simon, S. Thompson, T.A. Springer, Mapping of antigenic and functional epitopes on the α- and β-subunits of two related mouse glycoproteins involved in cell interactions, LFA-1 and Mac-1, J. Exp. Med. 158 (1983) 586–602.
[34] M. Patarroyo, P.G. Beatty, J.W. Fabre, C.G. Gahmberg, Identification of a cell surface protein complex mediating phorbol ester-induced adhesion (binding) among human mononuclear leukocytes, Scand. J. Immunol. 22 (1985) 171–182.
[35] T.-L. Lau, A.W. Partridge, M.H. Ginsberg, T.S. Ulmer, Structure of the integrin β3 transmembrane segment in phospholipid bicelles and detergent micelles, Biochemistry 47 (2008) 4008–4016.
[36] T.-L. Lau, V. Dua, T.S. Ulmer, Structure of the integrin αIIb transmembrane segment, J. Biol. Chem. 283 (2008) 16162–16168.
[37] P.E. Hughes, F. Diaz-Gonzalez, L. Leong, C. Wu, J.A. McDonald, S.J. Shattil, M.H. Ginsberg, Breaking the integrin hinge. A defined structural constraint regulates integrin signaling, J. Biol. Chem. 271 (1996) 6571–6574.
[38] O. Vinogradova, A. Velyvis, A. Velyviene, B. Hu, T.A. Haas, E.F. Plow, J. Qin, A structural mechanism of integrin, $\alpha_{IIb}\beta_3$ "inside-out" activation as regulated by its cytoplasmic face, Cell 110 (2002) 587–597.
[39] K.R. MacKenzie, J.H. Prestegard, D.M. Engelman, A transmembrane helixdimer , structure and implications, Science 276 (1997) 131–133.
[40] J.M. Cuthbertson, P.J. Bond, M.S.P. Sansom, Transmembrane helix–helix interactions: comparative simulations of the glycophorin A dimer, Biochemistry 45 (2006) 14298–14310.
[41] C. Kim, T.-L. Lau, T.S. Ulmer, M.H. Ginsberg, Interactions of platelet integrin α_{IIb} and β_3 transmembrane domains in mammalian cell membranes and their role in integrin activation, Blood (2009), doi:10.1182/blood-2008-10-186551.
[42] A. Vararattanavech, X. Lin, J. Torres, S.-M. Tan, Disruption of the integrin αLβ2 transmembrane domain interface by β2 Thr686 mutation activates αLβ2 and promotes micro-clustering of the αL subunits, J. Biol. Chem. 284 (2009) 3239–3249.
[43] Y.-Q. Ma, J. Yang, M.M. Pesho, O. Vinogradova, J. Qin, E.F. Plow, Regulation of integrin $\alpha_{IIb}\beta_3$ activation by distinct regions of its cytoplasmic tails, Biochemistry 45 (2006) 6656–6662.
[44] O. Vinogradova, J. Vaynberg, X. Kong, T.A. Haas, E.F. Plow, J. Qin, Membrane-mediated structural transitions at the cytoplasmic face during integrin activation, Proc. Natl. Acad. Sci. U. S. A. 101 (2004) 4094–4099.
[45] A. Bhunia, X.-Y. Tang, H. Mohanram, S.-M. Tan, S. Bhattacharjya, NMR solution conformations and interactions of integrin αLβ2 cytoplasmic tails, J. Biol. Chem. 284 (2009) 3873–3884.
[46] T.E. O'Toole, D. Mandelman, J. Forsyth, S.J. Shattil, E.F. Plow, M.H. Ginsberg, Modulation of the affinity of integrin αIIbβ3 (GPIIb-IIIa) by the cytoplasmic domain of αIIb, Science 254 (1991) 845–847.
[47] T.E. O'Toole, Y. Katagiri, R.J. Faull, K. Peter, R. Tamura, V. Quaranta, J.C. Loftus, S.J. Shattil, M.H. Ginsberg, Integrin cytoplasmic domains mediate inside-out signal transduction, J. Cell Biol. 124 (1994) 1047–1059.
[48] A. Czuchra, H. Meyer, K.R. Legate, C. Brakebusch, R. Fässler, Genetic analysis of β1 integrin "activation motifs" in mice, J. Cell Biol. 174 (2006) 889–899.
[49] M. Semmrich, A. Smith, C. Feterowski, S. Beer, B. Engelhardt, S.H. Busch, B. Bartsch, M. Laschinger, N. Hogg, K. Pfeffer, B. Holzmann, Importance of integrin LFA-1 deactivation for the generation of immune responses, J. Exp. Med. 201 (2005) 1987–1998.
[50] J. Ylänne, J. Huuskonen, T.E. O'Toole, M.H. Ginsberg, I. Virtanen, C.G. Gahmberg, Mutation of the cytoplasmic domain of the integrin β3 subunit: differential effects on cell spreading, recruitment to adhesion plaques, endocytosis and phagocytosis, J. Biol. Chem. 270 (1995) 9550–9557.
[51] M.D. Pierschbacher, E. Ruoslahti, Cell attachment activity of fibronectin can be duplicated by small synthetic fragments of the molecule, Nature 309 (1984) 30–33.
[52] E. Ruoslahti, M.D. Pierschbacher, New perspectives in cell adhesion: RGD and integrins, Science 238 (1987) 491–497.
[53] R.J. Gould, M.A. Polokoff, P.A. Friedman, T.F. Huang, J.C. Holt, J.J. Cook, S. Niewiarowski, Disintegrins: a family of integrin inhibitory proteins from viper venoms, Exp. Biol. Med. 195 (1990) 168–171.
[54] R. Rothlein, M.L. Dustin, S.D. Marlin, T.A. Springer, A human intercellular adhesion molecule (ICAM-1) distinct from LFA-1, J. Immunol. 137 (1986) 1270–1274.
[55] M. Patarroyo, E.A. Clark, J. Prieto, C. Kantor, C.G. Gahmberg, Identification of a novel adhesion molecule in human leukocytes by monoclonal antibody LB-2, FEBS Lett. 210 (1987) 127–131.
[56] C.G. Gahmberg, L. Valmu, S. Fagerholm, P. Kotovuori, E. Ihanus, L. Tian, T. Pessa-Morikawa, Leukocyte integrins and inflammation, Cell. Mol. Life Sci. 54 (1998) 549–555.
[57] D.E. Staunton, M.L. Dustin, T.A. Springer, Functional cloning of ICAM-2, a cell adhesion ligand for LFA-1 homologous to ICAM-1, Nature 339 (1989) 61–64.
[58] P. Nortamo, R. Salcedo, T. Timonen, M. Patarroyo, C.G. Gahmberg, A monoclonal antibody to the human leukocyte adhesion molecule intercellular adhesion molecule-2. Cellular distribution and molecular characterization of the antigen, J. Immunol. 146 (1991) 2530–2535.
[59] P. Nortamo, R. Li, R. Renkonen, T. Timonen, J. Prieto, M. Patarroyo, C.G. Gahmberg, The expression of human intercellular adhesion molecule-2 is refractory to inflammatory cytokines, Eur. J. Immunol. 21 (1991) 2629–2632.
[60] R. Renkonen, T. Paavonen, P. Nortamo, C.G. Gahmberg, Expression of endothelial adhesion molecules in vivo. Increased endothelial ICAM-2 expression in lymphoid malignancies, Am. J. Pathol. 140 (1992) 763–767.
[61] J. Fawcett, C.L.L. Holness, L.A. Needham, H. Turley, K.C. Gatter, D.Y. Mason, D.L. Simmons, Molecular cloning of ICAM-3, a third ligand for LFA-1, constitutively expressed on resting leukocytes, Nature 360 (1992) 481–484.
[62] R. Vazeux, P.A. Hoffman, J.K. Tomita, E.S. Dickinson, R.L. Jasman, T. St. John, W.M. Gallatin, Cloning and characterization of a new intercellular adhesion molecule ICAM-R, Nature 360 (1992) 485–488.
[63] P. Bailly, E. Tontti, P. Hermand, J.-P. Cartron, C.G. Gahmberg, The red cell LW blood group protein is an intercellular adhesion molecule which binds to CD11/CD18 leukocyte integrins, Eur. J. Immunol. 25 (1995) 3316–3320.
[64] A. Toivanen, E. Ihanus, M. Mattila, H.U. Lutz, C.G. Gahmberg, Importance of molecular studies on major blood groups–intercellular adhesion molecule-4, a blood group antigen involved in multiple cellular interactions, Biochim. Biophys. Acta 1780 (2008) 456–466.
[65] Y. Yoshihara, S. Oka, Y. Nemoto, Y. Watanabe, S. Nagata, H. Kagamiyama, K. Mori, An ICAM-related neurone glycoprotein, telencephalin, with brain segment-specific expression, Neuron 12 (1994) 541–553.
[66] L. Tian, Y. Yoshihara, T. Mizuno, K. Mori, C.G. Gahmberg, The neuronal glycoprotein telencephalin is a cellular ligand for the CD11a/CD18 leukocyte integrin, J. Immunol. 158 (1997) 928–936.
[67] L. Tian, J. Lappalainen, H. Nyman, P. Kilgannon, Y. Yoshihara, K. Mori, L.C. Andersson, S. Kaukinen, H. Rauvala, W.M. Gallatin, C.G. Gahmberg, Intercellular adhesion molecule-5 induces dendritic outgrowth by homophilic adhesion, J. Cell Biol. 150 (2000) 243–252.
[68] L. Tian, M. Stefanidakis, L. Ning, P. Van Lint, H. Nyman-Huttunen, C. Libert, S. Itohara, M. Mishina, H. Rauvala, C.G. Gahmberg, Activation of NMDA receptors promotes dendritic spine development through MMP-mediated ICAM-5 cleavage, J. Cell Biol. 178 (2007) 687–700.
[69] L. Tian, M. Autero, S. Hänninen, H. Rauvala, C.G. Gahmberg, Shedded neuronal ICAM-5 suppresses T-cell activation, Blood 111 (2008) 3615–3625.
[70] C.G. Gahmberg, L. Tian, L. Ning, H. Nyman-Huttunen, ICAM-5 — a novel two-facetted adhesion molecule in the mammalian brain, Immunol. Lett. 117 (2008) 131–135.
[71] G. Borriello, L. Prosperini, A. Luchetti, C. Pozzilli, Natalizumab treatment in pediatric multiple sclerosis: a case report, Eur. J. Paediat. Neurol. 13 (2009) 67–71.
[72] M. Lebwohl, S.K. Tyring, T.K. Hamilton, D. Toth, S. Glazer, N.H. Tawfik, P. Walicke, W. Dummer, X. Wang, M.R. Garovoy, D. Pariser, A novel targeted T-cell modulator, efalizumab, for plaque psoriasis, N. Engl. J. Med. 349 (2003) 2004–2013.
[73] P.S. Frenette, Locking a leukocyte integrin with statins, New Engl. J. Med. 345 (2001) 1419–1421.
[74] G. Weitz-Schmidt, K. Welzenbach, V. Brinkmann, T. Kamata, J. Kallen, C. Bruns, S. Cottens, Y. Takada, U. Hommel, Statins selectively inhibit leukocyte function

antigen-1 by binding to a novel regulatory integrin site, Nature Med. 7 (2001) 687–692.
[75] P. Nortamo, M. Patarroyo, C. Kantor, J. Suopanki, C.G. Gahmberg, Immunological mapping of the human leukocyte adhesion glycoprotein GP90 (CD18) by monoclonal antibodies, Scand. J. Immunol. 28 (1988) 537–546.
[76] S.C. Fagerholm, T.J. Hilden, C.G. Gahmberg, P marks the spot: site-specific integrin phosphorylation regulates molecular interactions, Trends Biochem. Sci. 29 (2004) 504–512.
[77] T. Chavakis, M. Hussain, S.M. Kanse, G. Peters, R.G. Bretzel, J.-I. Flock, M. Herrmann, K.T. Preissner, *Staphylococcus aureus* extracellular adherence protein serves as anti-inflammatory factor by inhibiting the recruitment of host leukocytes, Nat. Med. 8 (2002) 687–693.
[78] C. Xie, P. Alcaide, B.V. Geisbrecht, D. Schneider, M. Herrmann, K.T. Preissner, F.W. Luscinskas, T. Chavakis, Suppression of experimental autoimmune encephalomyelitis by extracellular adherence protein of *Staphylococcus aureus*, J. Exp. Med. 203 (2006) 985–994.
[79] R. Li, P. Nortamo, L. Valmu, M. Tolvanen, C. Kantor, C.G. Gahmberg, A peptide from ICAM-2 binds to the leukocyte integrin CD11a/CD18 and inhibits endothelial cell adhesion, J. Biol. Chem. 268 (1993) 17513–17518.
[80] E. Koivunen, T.-M. Ranta, A. Annila, S. Taube, A. Uppala, M. Jokinen, G. van Willigen, E. Ihanus, C.G. Gahmberg, Inhibition of β_2 integrin-mediated leukocyte cell adhesion by leucine–leucine–glycine motif-containing peptides, J. Cell Biol. 153 (2001) 905–916.
[81] E.Y. Choi, E. Chavakis, M.A. Czabanka, H.F. Langer, L. Fraemohs, M. Economopoulou, R.K. Kundu, A. Orlandi, Y.Y. Zheng, D.A. Prieto, C.M. Ballantyne, S.L. Constant, W.C. Aird, T. Papayannopoulou, C.G. Gahmberg, M.C. Udey, P. Vajkoczy, T. Quertermous, S. Dimmeler, C. Weber, T. Chavakis, Del-1, an endogenous leukocyte-endothelial adhesion inhibitor, limits inflammatory cell recruitment, Science 322 (2008) 1101–1104.
[82] C. Hidai, T. Zupancic, K. Penta, A. Mikhail, M. Kawana, E.E. Quertermous, Y. Aoka, M. Fukagawa, Y. Matsui, D. Platika, R. Auerbach, B.L.M. Hogan, R. Snodgrass, T. Quertermous, Cloning and characterization of developmental endothelial locus-1: an embryonic endothelial cell protein that binds the $\alpha V\beta 3$ integrin receptor, Genes Dev. 12 (1998) 21–33.
[83] E.C. Butcher, Leukocyte-endothelial cell recognition: three (or more) steps to specificity and diversity, Cell 67 (1991) 1033–1036.
[84] T.A. Springer, Traffic signals on endothelium for lymphocyte recirculation and leukocyte emigration, Annu. Rev. Physiol. 57 (1995) 827–872.
[85] M.L. Dustin, A.S. Shaw, Costimulation: building an immunological synapse, Science 283 (1999) 649–650.
[86] H. Zhang, J.M. Casasnovas, M. Jin, J.-h. Liu, C.G. Gahmberg, T.A. Springer, J.-h. Wang, An unusual allosteric mobility of the C-terminal helix of a high-affinity α_L integrin I domain variant bound to ICAM-5, Mol. Cell 31 (2008) 432–437.
[87] J. Emsley, C.G. Knight, R.W. Farndale, M.J. Barnes, R.C. Liddington, Structural basis of collagen recognition by integrin $\alpha 2\beta 1$, Cell 101 (2000) 47–56.
[88] M. Cheng, S.-Y. Foo, M.-L. Shi, R.-H. Tang, L.-S. Kong, S.K.A. Law, S.-M. Tan, Mutation of a conserved asparagine in the I-like domain promotes constitutively active integrins $\alpha_L\beta_2$ and $\alpha_{IIb}\beta_3$, J. Biol. Chem. 282 (2007) 18225–18232.
[89] M. Kim, C.V. Carman, T.A. Springer, Bidirectional transmembrane signaling by cytoplasmic domain separation in integrins, Science 301 (2003) 1720–1725.
[90] M.A. Arnaout, S.L. Goodman, J.P. Xiong, Structure and mechanics of integrin-based cell adhesion, Curr. Opin. Cell Biol. 19 (2007) 495–507.
[91] D.R. Critchley, A.R. Gingras, Talin at a glance, J. Cell Sci. 121 (2008) 1345–1347.
[92] B. Garcia-Alvarez, J.M. de Pereda, D.A. Calderwood, T.S. Ulmer, D. Critchley, I.D. Campbell, M.H. Ginsberg, R.C. Liddingon, Structural determinants of integrin recognition by talin, Mol. Cell 11 (2003) 49–58.
[93] K.L. Wegener, A.W. Partridge, J. Han, A.R. Pickford, R.C. Liddington, M.H. Ginsberg, I.D. Campbell, Structural basis of integrin activation by talin, Cell 128 (2007) 171–182.
[94] S. Rodius, O. Chaloin, M. Moes, E. Schaffner-Reckinger, I. Landrieu, G. Lippens, M. Lin, J. Zhang, N. Kieffer, The talin rod IBS2 α-helix interacts with the $\beta 3$ integrin cytoplasmic tail membrane-proximal helix by establishing charge complementary salt bridges, J. Biol. Chem. 283 (2008) 24212–24223.
[95] B. Nieswandt, M. Moser, I. Pleines, D. Varga-Szabo, S. Monkley, D. Critchley, R. Fässler, Loss of talin1 in platelets abrogates integrin activation, platelet aggregation, and thrombus formation in vitro and in vivo, J. Exp. Med. 204 (2007) 3113–3118.
[96] H. Takala, E. Nurminen, S.M. Nurmi, M. Aatonen, T. Strandin, M. Takatalo, T. Kiema, C.G. Gahmberg, J. Ylänne, S.C. Fagerholm, $\beta 2$ integrin phosphorylation on Thr758 acts as a molecular switch to regulate 14-3-3 and filamin binding, Blood 112 (2008) 1853–1862.
[97] N. Watanabe, L. Bodin, M. Pandey, M. Krause, S. Coughlin, V.A. Boussiotis, M.H. Ginsberg, S.J. Shattil, Mechanisms and consequences of agonist-induced talin recruitment to platelet integrin $\alpha IIb\beta 3$, J. Cell Biol. 181 (2008) 1211–1222.
[98] S.M. Cifuni, D.D. Wagner, W. Bergmeier, CalDAG-GEFI and protein kinase C represent alternative pathways leading to activation of integrin $\alpha IIb\beta 3$ in platelets, Blood 112 (2008) 1696–1703.
[99] R. Pasvolsky, S.W. Feigelson, S.S. Kilic, A.J. Simon, G. Tal-Lapidot, V. Grabovsky, J.R. Crittenden, N. Amariglio, M. Safran, A.M. Graybiel, G. Rechavi, S. Ben-Dor, A. Etzioni, R. Alon, A LAD-III syndrome is associated with defective expression of the Rap-1 activator CalDAG-GEFI in lymphocytes, neutrophils, and platelets, J. Exp. Med. 204 (2007) 1571–1582.
[100] S. Ussar, H.-V. Wang, S. Linder, R. Fässler, M. Moser, The Kindlins: subcellular localization and expression during murine development, Exp. Cell Res. 312 (2006) 3142–3151.
[101] E. Montanez, S. Ussar, M. Schifferer, M. Bösl, R. Zent, M. Moser, R. Fässler, Kindlin-2 controls bidirectional signaling of integrins, Genes Dev. 22 (2008) 1325–1330.
[102] M. Moser, B. Nieswandt, S. Ussar, M. Pozgajova, R. Fässler, Kindlin-3 is essential for integrin activation and platelet aggregation, Nat. Med. 14 (2008) 325–330.
[103] L. Wang, W. Deng, T. Shi, D. Ma, URP2SF, a FERM and PH domain containing protein, regulates NF-kB and apoptosis, Biochem. Biophys. Res. Commun. 368 (2008) 899–906.
[104] A. Mory, S.W. Feigelson, N. Yarali, S.S. Kilic, G.I. Bayhan, R. Gershoni-Baruch, A. Etzioni, R. Alon, Kindlin-3: a new gene involved in the pathogenesis of LAD-III, Blood 112 (2008) 2591.
[105] T.W. Kuijpers, E. van de Vijver, M.A.J. Weterman, M. de Boer, A.T.J. Tool, T.K. van den Berg, M. Moser, M.E. Jakobs, K. Seeger, O. Sanal, S. Unal, M. Cetin, D. Roos, A.J. Verhoeven, F. Baas, LAD-1/variant syndrome is caused by mutations in FERMT3, Blood, doi:10.1182/blood-2008-10-182154.
[106] Y.-Q. Ma, J. Qin, C. Wu, E.F. Plow, Kindlin-2 (Mig-2): a co-activator of β_3 integrins, J. Cell Biol. 181 (2008) 439–446.
[107] R. Sampath, P.J. Gallagher, F.M. Pavalko, Cytoskeletal interactions with the leukocyte integrin $\beta 2$ cytoplasmic tail, J. Biol. Chem. 273 (1998) 33588–33594.
[108] P. Stanley, A. Smith, A. McDowall, A. Nicol, D. Zicha, N. Hogg, Intermediate-affinity LFA-1 binds α-actinin-1 to control migratio at the leading edge of the T cell, EMBO J. 27 (2008) 62–75.
[109] D.A. Calderwood, A. Huttenlocher, W.B. Kiosses, D.M. Rose, D.G. Woodside, M.A. Schwartz, M.H. Ginsberg, Increased filamin binding to β-integrin cytoplasmic domains inhibits cell migration, Nature Cell Biol. 3 (2001) 1060–1068.
[110] Y. Lad, T. Kiema, P. Jiang, O.T. Pentikäinen, C.H. Coles, I.D. Campbell, D.A. Calderwood, J. Ylänne, Structure of three tandem filamin domains reveals auto-inhibition of ligand binding, EMBO J. 26 (2007) 3993–4004.
[111] T. Kiema, Y. Lad, P. Jiang, C.L. Oxley, M. Baldassarre, K.L. Wegener, I.D. Campbell, J. Ylänne, D.A. Calderwood, The molecular basis of filamin binding to integrins and competition with talin, Mol. Cell 21 (2006) 337–347.
[112] W. Kolanus, Guanine nucleotide exchange factors of the cytohesin family and their roles in signal transduction, Immunol. Rev. 218 (2007) 102–113.
[113] C. Geiger, W. Nagel, T. Boehm, Y. van Kooyk, C.G. Figdor, E. Kremmer, N. Hogg, L. Zeitlmann, H. Dierks, K.S. Weber, W. Kolanus, Cytohesin-1 regulates beta-2 integrin-mediated adhesion through both ARF-GEF function and interaction with LFA-1, EMBO J. 19 (2000) 2525–2536.
[114] U. Korthauer, W. Nagel, E.M. Davis, M.M. Le Beau, R.S. Menon, E.O. Mitchell, C.A. Kozak, W. Kolanus, J.A. Bluestone, AnergicT , lymphocytes selectively express an integrin regulatory protein of the cytohesin family, J. Immunol. 164 (2000) 308–318.
[115] S.R. Frank, J.C. Hatfield, J.E. Casanova, Remodeling of the actin cytoskeleton is coordinately regulated by protein kinase C and the ADP-ribosylation factor nucleotide exchange factor ARNO, Mol. Biol. Cell 9 (1998) 3133–3146.
[116] L.C. Santy, K.S. Ravichandran, J.E. Casanova, The DOCK 180/Elmo complex couples ARNO-mediated Arf6 activation to the downstream activation of Rac1, Curr. Biol. 15 (2005) 1749–1754.
[117] K. Sendide, N.E. Reiner, J.S. Lee, S. Bourgoin, A. Talal, Z. Hmama, Cross-talk between CD14 and complement receptor 3 promotes phagocytosis of mycobacteria: regulation by phosphatidylinositol 3-kinase and cytohesin-1, J. Immunol. 174 (2005) 4210–4219.
[118] S.C. Fagerholm, T.J. Hilden, S.M. Nurmi, C.G. Gahmberg, Specific integrin α and β chain phosphorylations regulate LFA-1 activation through affinity-dependent and -independent mechanisms, J. Cell Biol. 171 (2005) 705–715.
[119] W. Zhang, Y. Shao, D. Fang, J. Huang, M.-S. Jeon, Y.-C. Liu, Negative regulation of T cell antigen receptor-mediated Crk-L-C3G signaling and cell adhesion by Cbl-b, J. Biol. Chem. 278 (2003) 23978–23983.
[120] E.Y. Choi, V.V. Orlova, S.C. Fagerholm, S.M. Nurmi, L. Zhang, C.M. Ballantyne, C.G. Gahmberg, T. Chavakis, Regulation of LFA-1-dependent inflammatory cell recruitment by Cbl-b and 14-3-3 proteins, Blood 111 (2008) 3607–3614.
[121] A. Wiedemann, J.C. Patel, J. Lim, A. Tsun, Y. Van Kooyk, E. Caron, Two distinct cytoplasmic regions of the β_2 integrin chain regulate RhoA function during phagocytosis, J. Cell Biol 172 (2006) 1069–1079.
[122] T.A. Chatila, R.S. Geha, M.A. Arnaout, Constitutive and stimulus-induced phosphorylation of CD11/CD18 leukocyte adhesion molecules, J. Cell Biol. 109 (1989) 3435–3444.
[123] J.P. Buyon, S.G. Slade, J. Reibman, S.B. Abramson, M.R. Philips, G. Weissmann, R. Winchester, Constitutive and induced phosphorylation of the α- and β-chains of the CD11/CD18 leukocyte integrin family, J. Immunol. 144 (1990) 191–197.
[124] L. Valmu, M. Autero, P. Siljander, M. Patarroyo, C.G. Gahmberg, Phosphorylation of the β-subunit of CD11/CD18 integrins by protein kinase C correlates with leukocyte adhesion, Eur. J. Immunol. 21 (1991) 2857–2862.
[125] M.L. Hibbs, S. Jakes, S.A. Stacker, R.W. Wallace, T.A. Springer, The cytoplasmic domain of the integrin lymphocyte function-associated antigen 1 β subunit: sites required for binding to intercellular adhesion molecule 1 and the phorbol ester-stimulated phosphorylation site, J. Exp. Med. 174 (1991) 1227–1238.
[126] L. Valmu, C.G. Gahmberg, Treatment with okadaic acid reveals strong threonine phosphorylation of CD18 after activation of CD11/CD18 leukocyte integrins with phorbol esters or CD3 antibodies, J. Immunol. 155 (1995) 1175–1183.
[127] L. Valmu, T.J. Hilden, G. van Willigen, C.G. Gahmberg, Characterization of $\beta 2$ (CD18) integrin phosphorylation in phorbol ester-activated T lymphocytes, Biochem. J. 339 (1999) 119–125.
[128] S. Fagerholm, N. Morrice, C.G. Gahmberg, P. Cohen, Phosphorylation of the cytoplasmic domain of the integrin CD18 chain by protein kinase C isoforms in leukocytes, J. Biol. Chem. 277 (2002) 1728–1738.
[129] T.J. Hilden, L. Valmu, S. Kärkkäinen, C.G. Gahmberg, Threonine phosphorylation sites in the β_2 and β_7 leukocyte integrin polypeptides, J. Immunol. 170 (2003) 4170–4177.
[130] H. Umehara, A. Takashima, Y. Minami, E.T. Bloom, Signal transduction via

phosphorylated adhesion molecule, LFA-1β (CD18), is increased by culture of natural killer cells with IL-2 in the generation of lymphokine-activated killer cells, Int. Immunol. 5 (1993) 19–27.
[131] R. Garnotel, J.-C. Monboisse, A. Randoux, B. Haye, J.P. Borel, The binding of type I collagen to lymphocyte function-associated antigen (LFA) 1 integrin triggers the respiratory burst of human polymorphonuclear neutrophils, J. Biol. Chem. 270 (1995) 27495–27503.
[132] S.C. Fagerholm, M. Varis, M. Stefanidakis, T.J. Hilden, C.G. Gahmberg, α-Chain phosphorylation of the human leukocyte CD11b/CD18 (Mac-1) integrin is pivotal for integrin activation to bind ICAMs and leukocyte extravasation, Blood 108 (2006) 3379–3386.
[133] S. Nilsson, D. Kaniowska, C. Brakebusch, R. Fässler, S. Johansson, Threonine 788 in integrin subunit β1 regulates integrin activation, Exp. Cell Res. 312 (2006) 844–853.
[134] J. Han, D.M. Rose, D.G. Woodside, L.E. Goldfinger, M.H. Ginsberg, Integrin $\alpha_4\beta_1$-dependent T cell migration requires both phosphorylation and dephosphorylation of the α_4 cytoplasmic domain to regulate the reversible binding of paxillin, J. Biol. Chem. 278 (2003) 34845–34853.
[135] C.J. Lim, K.H. Kain, E. Tkachenko, L.E. Goldfinger, E. Gutierrez, M.D. Allen, A. Groisman, J. Zhang, M.H. Ginsberg, Integrin-mediated protein kinase A activation at the leading edge of migrating cells, Mol. Biol. Cell 19 (2008) 4930–4941.
[136] D.A. Law, F.R. DeGuzman, P. Heiser, K. Ministri-Madrid, N. Killeen, D.R. Phillips, Integrin cytoplasmic tyrosine motif is required for outside-in alphaIIbβ3 signalling and platelet function, Nature 401 (1999) 808–811.
[137] X. Xi, P. Flevaris, A. Stojanovic, A. Chishti, D.R. Phillips, S.C.T. Lam, X. Du, Tyrosine phosphorylation of the integrin β_3 subunit regulates β_3 cleavage by calpain, J. Biol. Chem. 281 (2006) 29426–29430.
[138] B. Butler, M.P. Williams, S.D. Blystone, Ligand-dependent activation of integrin $\alpha_v\beta_3$, J. Biol. Chem. 278 (2003) 5264–5270.
[139] A. Datta, F. Huber, D. Boettiger, Phosphorylation of β_3 integrin controls ligand binding strength, J. Biol. Chem. 277 (2002) 3943–3949.
[140] Y. Lad, P. Jiang, S. Ruskamo, D.S. Harburger, J. Ylänne, I.D. Campbell, D.A. Calderwood, Structural basis of the migfilin–filamin interaction and competition with integrin β tails, J. Biol. Chem. 283 (2008) 35154–35163.
[141] S.S. Ithychanda, M. Das, Y.-Q. Ma, K. Ding, X. Wang, S. Gupta, C. Wu, E.F. Plow, J. Qin, Migfilin, a molecular switch in regulation of integrin activation, J. Biol. Chem. 284 (2009) 4713–4722.
[142] C.L. Oxley, N.J. Anthis, E.D. Lowe, I. Vakonakis, I.D. Campbell, K.L. Wegener, An integrin phosphorylation switch. The effect of β3 integrin tail phosphorylation on Dok1 and talin binding, J. Biol. Chem. 283 (2008) 5420–5426.
[143] S.M. Nurmi, M. Autero, A.K. Raunio, C.G. Gahmberg, S.C. Fagerholm, Phosphorylation of the LFA-1 integrin β_2-chain on Thr-758 leads to adhesion, Rac-1/Cdc42 activation and stimulation of CD69 expression in human T cells, J. Biol. Chem. 282 (2007) 968–975.
[144] A.B. Jaffe, A. Hall, Rho GTPases: biochemistry and biology, Annu. Rev. Cell Dev. Biol. 21 (2005) 247–269.
[145] M.A. del Pozo, M.A. Schwartz, J.R. Hu, W.B. Kiosses, A. Altman, M. Villalba, Guanine exchange-dependent and -independent effects of Vav1 on integrin-induced T cell spreading, J. Immunol. 170 (2003) 41–47.
[146] M.A. Martinez Gakidis, X. Cullere, T. Olson, J.L. Wilsbacher, B. Zhang, S.L. Moores, K. Ley, W. Swat, T. Mayadas, J.S. Brugge, Vav GEFs are required for β_2 integrin-dependent functions of neutrophils, J. Cell Biol. 166 (2004) 273–282.
[147] Y. Miyamoto, J. Yamauchi, A. Tanoue, C. Wu, W.C. Mobley, TrkB binds and tyrosine-phosphorylates Tiam1, leading to activation of Rac1 and induction of changes in cellular morphology, Proc. Natl. Acad. Sci. U. S. A. 103 (2006) 10444–10449.
[148] I.H.L. Hamelers, C. Olivo, A.E.E. Mertens, D.M. Pegtel, R.A. van der Kammen, A. Sonnenberg, J.G. Collard, The Rac activator Tiam 1 is required for α3β1-mediated laminin-5 deposition, cell spreading, and cell migration, J. Cell Biol. 171 (2005) 871–881.
[149] A. Gérard, R.A. van der Kammen, H. Janssen, S.I. Ellenbroek, J.G. Collard, The Rac activator Tiam1 controls efficient T-cell trafficking and route of trans-endothelial migration, Blood (2009), doi:10.1182/blood-2008-7-167668 prepublished online.
[150] S. Etienne-Manneville, A. Hall, Integrin-mediated activation of Cdc42 controls cell polarity in migrating astrocytes through PKCζ, Cell 106 (2001) 489–498.
[151] O.D. Perez, D. Mitchell, G.C. Jager, S. South, C. Murriel, J. McBride, L.A. Herzenberg, S. Kinoshita, G.P. Nolan, Leukocyte functional antigen 1 lowers T cell activation thresholds and signaling through cytohesin-1 and Jun-activating binding protein 1, Nat. Immunol. 4 (2003) 1083–1092.
[152] E. Sebzda, M. Bracke, T. Tugal, N. Hogg, D.A. Cantrell, Rap1A positively regulates T cells via integrin activation rather than inhibiting lymphocyte signaling, Nature Immunol. 3 (2002) 251–258.
[153] J.L. Dunne, R.G. Collins, A.L. Beaudet, C.M. Ballantyne, K. Ley, Mac-1, but not LFA-1, uses intercellular adhesion molecule-1 to mediate slow leukocyte rolling in TNF-α-induced inflammation, J. Immunol. 171 (2003) 6105–6111.
[154] K. Katagiri, A. Maeda, M. Shimonaka, T. Kinashi, RAPL, a Rap 1-binding molecule that mediates Rap1-induced adhesion through spatial regulation of LFA-1, Nat. Immunol. 4 (2003) 741–748.
[155] K. Katagiri, M. Imamura, T. Kinashi, Spatiotemporal regulation of the kinase Mst1 by binding protein RAPL is critical for lymphocyte polarity and adhesion, Nat. Immunol. 7 (2006) 919–928.
[156] A. Gérard, A.E.E. Mertens, R.A. van der Kammen, J.G. Collard, The Par polarity complex regulates Rap1- and chemokine-induced T cell polarization, J. Cell Biol. 176 (2007) 863–875.
[157] S.J. Hyduk, J. Oh, H. Xiao, M. Chen, M.I. Cybulsky, Paxillin selectively associates with constitutive and chemoattractant-induced high-affinity α4β1 integrins: implications for integrin signaling, Blood 104 (2004) 2818–2824.
[158] F.C. Guschiken, V. Patel, Y. Liu, S. Pradhan, A.L. Bergeron, Y. Peng, K.V. Vijayan, Protein phosphatase 2A negatively regulates integrin $\alpha_{IIb}\beta_3$ signaling, J. Biol. Chem. 283 (2008) 12862–12869.
[159] J. Ivaska, L. Nissinen, N. Immonen, J.E. Eriksson, V.-M. Kähäri, J. Heino, Integrin α2β1 promotes activation of protein phosphatase 2A and dephosphorylation of Akt and glycogen synthase kinase 3β, Mol. Cell. Biol. 22 (2002) 1352–1359.
[160] E. Mattila, T. Pellinen, J. Nevo, K. Vuoriluoto, A. Arjonen, J. Ivaska, Negative regulation of EGFR signalling through integrin-$\alpha_1\beta_1$-mediated activation of protein tyrosine phosphatase TCPTP, Nat. Cell Biol 7 (2005) 78–85.

Glossary

LFA-1: leukocyte function associated antigen ($\alpha_L\beta_2$, CD11a/CD18)
ICAM: intercellular adhesion molecule
Mac-1: macrophage antigen-1 ($\alpha_M\beta_2$, *CD11b/CD18*)
I-domain: intervening domain (A-domain)
MMP: matrix metalloprotease
LAD: leukocyte adhesion deficiency