Beta-1 integrin variants in myogenesis and cytoskeletal signaling
van der Fliers, A.

Link to publication

Citation for published version (APA):
van der Fliers, A. (2001). Beta-1 integrin variants in myogenesis and cytoskeletal signaling

General rights
It is not permitted to download or to forward/distribute the text or part of it without the consent of the author(s) and/or copyright holder(s), other than for strictly personal, individual use, unless the work is under an open content license (like Creative Commons).

Disclaimer/Complaints regulations
If you believe that digital publication of certain material infringes any of your rights or (privacy) interests, please let the Library know, stating your reasons. In case of a legitimate complaint, the Library will make the material inaccessible and/or remove it from the website. Please Ask the Library: http://uba.uva.nl/en/contact, or a letter to: Library of the University of Amsterdam, Secretariat, Singel 425, 1012 WP Amsterdam, The Netherlands. You will be contacted as soon as possible.
CHAPTER 1

General introduction
General introduction

During development multicellular organisms and tissues are formed and cells are held together by adhesion molecules. Adhesion between cells and between cells and the extracellular matrix is mediated by different members of the integrin, cadherin, immunoglobulin, and selectin families [1]. Besides their role in the maintenance of tissue structure and polarity, cell adhesion molecules are also involved in the regulation of cell proliferation and differentiation [2, 3].

The work described in this thesis focuses on a variant of the cytoplasmic domain of the β1 integrin subunit. We determined the expression pattern and cytoskeletal interactions specific for this variant. Furthermore, we studied the epithelial-mesenchymal-like transition that follows upon β1 expression in epithelial cells that lack endogenous β1. In this chapter, the integrin protein family and their splice variants will be introduced. Several aspects of the biological function of integrins in general and in myogenesis in particular will be addressed.

Integrin protein family, extracellular matrix and ligands

Integrins are a family of glycosylated, heterodimeric transmembrane adhesion receptors that consist of a non-covalently bound α- and β-subunit. Most integrins bind to extracellular matrix (ECM) components while others bind to receptors on other cells. The name integrin refers to their function in the integral linkage of the cells’ exterior (ECM) to the cells’ interior (cytoskeleton). By reason of their fundamental role in cell adhesion, migration and signal transduction, integrins play an important role in various processes such as immune function, platelet aggregation, tissue repair, invasion and metastasizing of cancer cells. Integrins are highly conserved in the taxonomy from sponges to humans, although the complexity of variants and their redundancy increases later in taxonomy [4, 5]. To date 18 α and 8 β-subunits have been identified in vertebrates, which form at least twenty-four different heterodimers. (Table I) (see for recent review [6]). The combination of the α and β-subunit determines the ligand specificity of the integrins. Many integrins have binding specificities for the same ligands (see Table I) and it is the combination of the integrin expression/activation pattern and the available ligand that determines the in vivo binding.

The extracellular matrix (ECM) is a complex network of high molecular weight proteins (e.g. laminins, collagens, fibronectin) and polysaccharides, which are deposited by the surrounding cells [7]. The ECM provides structural support for cells but also acts as a physical barrier or selective filter to soluble molecules (e.g. basement membrane). Finally, ECMs sequester growth factors and are critical for the differentiation and growth of a number of cell types. This latter phenomenon is also known as anchorage-dependent growth.

Integrins containing the α4, α5, α8, αIβ and αv subunits bind to ECM components that contain the RGD (Arg-Gly-Asp) sequence as present in fibronectin. Other integrins bind to laminins and collagens, of which the cryptic RGD sites are thought to be normally inaccessible for binding. In general, integrins from haematopoietic cells bind to receptors on other cells (e.g. ICAMs and VCAMs) or plasma proteins that are deposited at sites of injury (e.g. fibrinogen, von Willebrand factor) and complement factors (e.g. iC3b). Several pathogenic agents bind to integrins, such as snake venoms (disintegrins) which prevent platelet-mediated blood plug formation. Furthermore, micro-organisms (Lyme disease spirochete, Borrelia burgdorferi; Yersinia invasin protein) and viruses (echo-; adeno-; papilloma-; foot-and-mouth-virus; HIV Tat protein) use the integrins as their
Table I
Integrins and their ligands

<table>
<thead>
<tr>
<th>Integrin</th>
<th>ECM</th>
<th>Soluble</th>
<th>Ligand Cell-cell</th>
<th>Pathogen</th>
</tr>
</thead>
<tbody>
<tr>
<td>α1 β1</td>
<td>Co; Ln</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>α2 β1</td>
<td>Co; Ln</td>
<td>-</td>
<td>-</td>
<td>echovirus 1</td>
</tr>
<tr>
<td>α3 β1</td>
<td>Ln; Co; Ln</td>
<td>-</td>
<td>-</td>
<td><em>Yersinia</em> (invasin)</td>
</tr>
<tr>
<td>α4 β1</td>
<td>Fn</td>
<td>pp-vWF, tTG; FXIII</td>
<td>VCAM-1</td>
<td><em>Yersinia</em> (invasin)</td>
</tr>
<tr>
<td>β7</td>
<td>Fn</td>
<td>-</td>
<td>VCAM-1; MadCAM</td>
<td>-</td>
</tr>
<tr>
<td>α5 β1</td>
<td>Fn</td>
<td>tTG</td>
<td>ADAM15</td>
<td><em>Yersinia; Borrelia; Shigella</em> (Ipa)</td>
</tr>
<tr>
<td>α6 β1</td>
<td>Ln</td>
<td>-</td>
<td>ADAM1,2,9</td>
<td>papilloma virus; <em>Yersinia</em> (invasin)</td>
</tr>
<tr>
<td>β4</td>
<td>Ln</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>α7 β1</td>
<td>Ln</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>α8 β1</td>
<td>Fn; Tn; Nn</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>α9 β1</td>
<td>Tn; Op; Co; Ln</td>
<td>pp-vWF; tTG; FXIII</td>
<td>VCAM-1; ADAM-12,15</td>
<td>-</td>
</tr>
<tr>
<td>α10 β1</td>
<td>Co</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>α11 β1</td>
<td>Co</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>α7 β1</td>
<td>Fn; Vn</td>
<td>-</td>
<td>-</td>
<td>echovirus 22</td>
</tr>
<tr>
<td>β3</td>
<td>Vn; Tn; Fg; vWF</td>
<td>ADAM-15, FGF-2, vWF</td>
<td>-</td>
<td>snake venoms, disintegrins;</td>
</tr>
<tr>
<td></td>
<td>Fg; Op; Tn; Tsp; Op; Co; Ln; Fg;</td>
<td>MMP2</td>
<td>-</td>
<td>adenovirus; HIV Tat</td>
</tr>
<tr>
<td>β5</td>
<td>Vn</td>
<td>-</td>
<td>-</td>
<td>adenovirus 2, HIV Tat</td>
</tr>
<tr>
<td>β6</td>
<td>Fn; Tn</td>
<td>TGFβ-LAP</td>
<td>-</td>
<td>foot-and-mouth disease virus</td>
</tr>
<tr>
<td>β8</td>
<td>Co; Ln; Fn</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>α11bβ3</td>
<td>Vn; Fn</td>
<td>Fg; vWF</td>
<td>-</td>
<td><em>Borrelia</em> disintegrins;</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Ticks; Lecch (decorsin, ornatin)</td>
</tr>
<tr>
<td>αL β2</td>
<td>-</td>
<td>ICAM1-6</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>αM β2</td>
<td>-</td>
<td>Fg;iC3b; FX</td>
<td>ICAM1; VCAM6</td>
<td><em>Candida; Borrelia.</em></td>
</tr>
<tr>
<td>αX β2</td>
<td>-</td>
<td>Fg; iC3b</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>αD β2</td>
<td>-</td>
<td>-</td>
<td>ICAM3,6; VCAM1</td>
<td>-</td>
</tr>
<tr>
<td>αE β3</td>
<td>-</td>
<td>-</td>
<td>E-cadherin</td>
<td>-</td>
</tr>
</tbody>
</table>


receptors (reviewed in [8]). Interaction between α6β1 on the egg cell and ADAM-1 and -2 (A Disintegrin And Metallo-protease) on sperm cells has been implicated in egg-sperm cell fusion [9, 10] and other ADAMs have also been shown to interact with integrins.

Despite the overlap in binding specificity of many of the integrins, the loss of nearly each integrin α- or β-subunit leads to biological defects as shown in genetically modified mice. These gene knock-out studies show that the loss of most integrin subunits is lethal at embryonic stages or shortly after birth (reviewed in [11-13]).
Integrins in disease

Mutations in the integrin β2-subunit lead to leukocyte adhesion deficiency (LAD1), and mutations in either the α1βb or β3-subunit result in the bleeding disease Glanzmann's thrombasthenia [14]. Junctional epidermolysis bullosa associated with pyloric atresia (PA-JEB), a skin blistering disease, is due to mutations in genes encoding either the α6 or β4 integrin subunit [15-17]. Loss of the α6β4 integrin results in rudimentary hemidesmosomes and subsequent detachment of the epidermis from its basal lamina upon physical stress [18, 19]. Similarly, mutations in the α7 integrin subunit have been found to result in a mild form of progressive muscular dystrophy [20, 21]. Alternatively, reduced levels of laminin (α2-chain) in certain muscular dystrophies result in decreased levels of the α7β1 integrin [22-24].

Finally, integrins play an important role in various aspects of cancer. Integrin expression patterns are altered in metastatic cells, resulting in changes of cell adhesion and migration which often lead to the detachment of cells from the primary tumor, migration and invasion into the target organ. These metastatic cells gain the capacity to survive without adhesive cues also named anchorage independent growth. In addition, integrins play a role in the neovascularization of tumors, that provides the tumor cells with the necessary nutrients for proliferation [25, 26].

Integrin structure

The ligand-binding site of integrins is present in the globular head domain formed by the α and β-chains, while the rest of the extracellular domain forms the membrane-proximal stalk-like structure. The small cytoplasmic tails are approximately 30-50 amino acids long and do not contain catalytic or consensus protein-protein binding motifs. An exception is the cytoplasmic domain of β4, that contains two pairs of fibronectin-III repeats and is approximately 1000 amino acids long. The extracellular domains of several integrin α-subunits (i.e. the collagen binding α1, α2, α10, α11, the E-cadherin binding αE and the leukocyte integrins αD αX, αM, αL) contain a 200 amino acid insertion, the I domain. Structural analysis of integrin I domains have revealed the presence of a characteristic metal ion-dependent (Mg²⁺) adhesion site motif (MIDAS) shown to be critical for ligand binding [6, 27]. A similar I domain is predicted to be present in the extracellular part of the β-chains [28]. Comparisons of structure have suggested that the seven repeat motifs, present in the extracellular domain of all the α-subunits, fold into a seven-bladed propeller structure that forms one globular domain with the ligand-binding site on the upper surface [29]. The optional I domain is inserted between blades number two and three of this propeller and the divergent cation-binding sites are located on the lower surface of blades 4-7 [30]. Besides glycosylation of both the α- and β-subunits, post-translational cleavage of α-subunits, that lack an I domain results in a disulfide-linked heavy and light chain. The noncleaved α4-subunit forms an exception to this rule. Post-translational cleavage has been implicated in the modulation of the affinity of α6 integrins [31].

Integrin activation and deactivation: inside-out signaling

The affinities of integrins for their ligands may vary depending on the cell type in which they are expressed [32-34]. Leukocyte and platelet integrins require activation induced by agonist (i.e thrombin, epinephrin) for binding to their ligand. This inside-out signaling has been extensively studied in platelets and requires the activity of protein kinase-C (PKC) and Rho-like GTPases.
Chapter 1

The current model is that the cytoplasmic domain of the α-subunit inhibits, directly or indirectly, the interaction of the β-subunit with cytoskeletal components. This inhibition is relieved upon binding of a ligand to an integrin or by signaling induced by another agonist possibly by inducing a conformational change which either splits or slides the α- and β-cytoplasmic tails relatively to each other [35, 36]. Mutation and deletion studies have indicated a crucial role of the conserved α (GFFKR) and β (LLxxxihDR) membrane proximal sequences. Changes in integrin affinity (interaction of a single integrin with its ligand) are the result of conformational changes in the integrins, which can be detected by antibodies that identify activation dependent neo-epitopes. Certain cations (e.g. Mn²⁺) and antibodies may activate integrins by stabilizing or inducing their active conformation [32]. Integrin-ligand binding is also increased by avidity. The concerted interaction of clustered receptors with a ligand can be induced by multiple binding sites in the ligand (polyvalent) and/or by cytoskeletal interactions that organize integrin clusters from within the cell. For example, in leukocytes it has been found that the integrin subunit β2 first needs to be released from talin in the cytoskeleton, before it can become clustered and bind ligand. Subsequently the integrin associates with α-actinin, which leads to a stable interaction with the cytoskeleton [37].

In adherent cells, integrins are apparently in a constitutive active conformation. Phosphorylation of integrin cytoplasmic domains [38, 39] and proteolytic cleavage [40-42] have been suggested to affect integrin-ligand binding. However, there is no consensus as yet on the general mechanism that regulates integrin-mediated cell adhesion. Cell migration is a process that requires the precise regulation of integrin-mediated adhesion/release [43]. The repeated cycle of the assembly and disassembly of adhesive complexes (focal adhesions) at the cells’ front, where the polymerization of actin drives the protrusion of the cell membrane, cytoskeletal contraction and detachment of the rear results in a net displacement of the cell. It has been found in migrating fibroblast that a pool of integrin surface molecules is internalized and recycled to the cells posterior [44-46]. Alternately, ligand-bound integrins in the rear of the cell are left as footprints on the underlying substrate [46, 47].

**Splice variants of integrins**

Alternative mRNA splicing leads to additional complexity of the integrin family [48]. Variants of both the extracellular and cytoplasmic domains have been reported. Alternative extracellular domains may account for different ligand binding affinities or variations in the state of activation, while variants of the cytoplasmic domain may modulate integrin activity, cytoskeletal associations and/or signaling events.

Extracellular variants of Drosophila αPS2 differ in their affinities for D-laminin [49]. However, no differences in laminin binding affinity of the extracellular domain variants of α6 (X1 or X1X2) have been reported [50, 51]. Activation and migration on different laminin isoforms of the variants of the α7 extracellular domain (X1, X2) was found to be regulated in a cell type specific manner [52, 53]. Similarly, a single amino acid polymorphism in the extracellular domain of β3 leads to a different state of platelet activation [54]. The biological relevance of two secreted, truncated products of the extracellular domains of β3 and αIIb remains to be determined [55, 56].

Variants of the cytoplasmic domain have been found of the three closely related laminin receptor α-chains: i.e. α3A, B; α6A, B; α7A, B, and C. Their sequences differ after the membrane proximal GFFKR motif, which is crucial for correct heterodimerization and the regulation of
binding activity of the integrins. Splicing of the mRNA of these laminin receptors is regulated in a developmentally and tissue specific manner and is conserved in men and mice. The homology between the A and B cytoplasmic variants of different α subunits is higher than that between the variants of the same α subunit [48]. During embryogenesis the first variants expressed are the α3B, α6B and α7B cytoplasmic variants, which later in development are replaced by the A variants of α subunits in many tissues. The function of this developmental regulated switching of variants is currently unknown. Notwithstanding the observation that α6A and α6B show differences with respect to phosphorylation and cell migration in vitro, no specific differences have been detected with respect to their ligand specificity and/or activation [57]. Similarly, no differences have yet been found for the variants of the cytoplasmic domain of α3 and α7 with respect to regulation, migration and ligand binding. Although the lack of α6 expression results in embryonic defects and death around birth due to severe blistering of the skin [58, 59], genetically modified mice that express only the α6B integrin variant have a normal phenotype [60]. The only effect detected in these mice is a reduction in the number of lymphocytes and a decreased migration of lymphocytes on fibronectin in vitro. Thus the switching from α6B to α6A appears not to be crucial for embryonic development [60].

Cytoplasmic variants of several integrin β-subunits have been described. The best studied are the five cytoplasmic variants of the β1 subunit, the most abundantly expressed integrin subfamily: β1A, B, C1, C2 and D (this thesis, [48]). Antibody inhibition studies and studies with mice in which the gene encoding β1 has been disrupted by homologous recombination have demonstrated the critical role of β1-integrins in development, cell differentiation, migration and the assembly of the extracellular matrix proteins [13]. β1A is present in all tissues, except cardiac and skeletal muscle, which instead express the β1D variant (this thesis). The β1B and β1C variants are minor forms and are present in man, but not in the mouse [61]. Both variants behave as inactive integrins, which is probably due to their failure to become localized at focal adhesions. Expression of β1B or β1C variant in cells decreases the ability of cells to adhere and to migrate on extracellular matrix components. Similarly, expression of β1B or β1C inhibits DNA synthesis and cell proliferation, whereas β1A does not inflict such inhibition [62-68].

The β1D isoform is very homologous to β1A. Both variants share the first 24 amino acids of their cytoplasmic domains and the two NPXY focal contact localization sequences (cyto-2 and -3 domains) in the C-terminus are also conserved. In non-muscle cells, transfected β1D is localized in focal contacts and activates focal adhesion kinase (FAK), MAP-Kinase and RhoA, similarly to β1A ([69, 70, 71], this thesis). However, Baudoin et al. [72] have shown that β1A and β1D are not functionally equivalent in embryonic development. The replacement of β1A by β1D results in abnormal migration of neuroepithelial cells and embryonic lethality in mice, despite the finding that reciprocal replacement in striated muscle of β1D by β1A does not lead to severe abnormalities in vivo [72].

Variants of the cytoplasmic domain of β3 (A-C) and β4 (A-E) have also been reported. The β3B variant is similar to β1B; it lacks the NPXY motifs, which results in the defective localization in focal adhesions and impaired phosphorylation of FAK. In addition expression of β3C in cells results in reduced adhesion and decreased cell survival [73]. The β4A-subunit is the most abundant β4 variant, while the function and characteristics of the other minor β4 variants remain to be elucidated.
Chapter 1

Studies using mutations, truncations or domain swaps of integrin cytoplasmic domains have indicated that the cytoplasmic tails of the integrin β subunits (i.e., the conserved NPXY/F motifs are required) contain sufficient information for localizing integrins into preexisting focal contacts, presumably by binding to cytoskeletal components enriched at those sites [74-77]. Furthermore, expression of β cytoplasmic domains, as chimeric proteins containing the extracellular and transmembrane domains of the interleukin-2 receptor (IL2R) at high levels, results in a dominant negative effect on focal contact formation and integrin-mediated signaling. This is probably due to the capturing of cytoplasmic focal contact components by the cytoplasmic domain. In addition, studies in the fruitfly have demonstrated that the cytoplasmic domain of integrin β-subunits is sufficient for correct localization [78].

Outside-in signaling

Adherent cells in cell culture form specialized structures, focal adhesions or focal contacts at sites where there is close contact between the plasma membrane and the underlying extracellular matrix and at which integrins, signaling and cytoskeletal molecules are colocalized (see Fig. 1). A hierarchy in the recruitment of focal adhesion components is observed upon integrin aggregation when the integrin is bound to ligand (receptor occupancy) [79, 80]. Furthermore there are differences in the constituents and the activation state of the different focal contacts even within the same cell [81-85]. The adaptor protein tensin and the tyrosine kinase FAK (focal adhesion kinase) are the first proteins to be recruited after integrins cluster. Ligand binding to the clustered integrins leads to the recruitment of cytoskeletal components such as, vinculin, talin and α-actinin. Furthermore, ligand binding and tension induces phosphorylation events at focal adhesions by members of the Src protein-kinase family including: cSrc, Csk, Fyn, which leads to the recruitment and activation of downstream signaling molecules e.g., activation of the Rho-like GTPases (this thesis), phospholipase-Cγ (PLCγ), and the ERK and JNK signaling cascades [86, 87]. Ultimately, this integrin signaling cascade leads to cytoskeletal rearrangements (this thesis), anchorage dependent completion of the cell cycle and integrin-mediated gene transcription [88]. The Rho family of GTPases play an important role in cytoskeletal rearrangements [89]. These proteins act as molecular switches, which are active when they are bound to GTP and inactive when bound to GDP. The GTP loading of Rho-like GTPases is regulated by GEFs (guanidine exchange factors), GAPs (GTPase-activating proteins) and GDIs (guanidine dissociating factors). As a key regulator of actomyosin-based contractility, RhoA plays a central role in the formation of both stress fibers and focal adhesions [90].

The formation of signaling complexes, which are modified and strengthened by multiple interactions between its components, is a recurring theme in integrin-mediated signaling. These stabilizing and modifying interactions make it difficult to define the regulation and crucial primary interactions in integrin-mediated signaling [91]. Hemidesmosomes form a separate class of cell-matrix adhesive structures found in the epidermis. Hemidesmosomes are linked to the intermediate filament system which involves the interaction of the cytoplasmic domain of β4 of the α6β4 integrin with associated proteins (plectin and BP230), this in contrast to the actin linkage in the other integrin-cytoskeletal associations [18, 92].
General introduction

**Figure 1. Schematic representation of a focal adhesion and an adherens-junction**

Transmembrane integrins bind to the extracellular matrix and associate within the cell to a complex of cytoskeletal proteins that link them to actin filaments. These focal adhesions form also the basis for the assembly of signaling complexes of which several components are indicated. The right sight of the figure shows an adherens-junction. The homophilic adhesion receptors, cadherins are linked via β-catenin and α-catenin to the actin cytoskeleton.

**Cross talk with integrins**

Integrins have been shown to affect the expression and activity of other integrins and different types of adhesion molecules (see also chapter 5). Occupancy of integrins by ligand has been shown to activate ligand binding of other integrins in a positive feed back loop [93]. Conversely, inhibition of one integrin by another is known as trans-dominant inactivation [53, 94-96]. Integrin-mediated signaling or potential changes in integrin-cytoskeletal interactions may account for these effects. Integrin-mediated adhesion can also modify cadherin-based intercellular adhesions [97]. A role of integrins in the down-regulation of cadherin activity during neural crest cell migration and epithelium-mesenchyme transitions (EMT) has been implicated, suggesting the existence of an opposite effect of cadherin and integrin-mediated adhesion. EMT occurs during specific stages of
Chapter 1

Embryonic development but also under certain pathological conditions as in the loss of epithelial polarity in breast tumor cells [98, 99].

Syndecans are transmembrane heparan sulfate proteoglycans whose external glycosaminoglycan chains bind ligands in the extracellular matrix. Syndecan-4 promotes integrin-mediated focal contact formation, probably by signaling through its cytoplasmic association with PKC and PIP$_2$ [100].

**Cytoplasmic proteins binding to integrins**

As mentioned above, the small cytoplasmic domains of integrins have no intrinsic catalytic capacity or protein-protein interaction motifs. Nevertheless, recent studies using yeast two-hybrid interaction assays and biochemical assays have identified an increasing number of integrin binding partners (see Table II and references therein, reviewed in [33, 34, 101-103]). These proteins can be grouped based on their proposed functions in three categories: 1) cytoskeletal proteins which anchor the integrins to the cytoskeleton and could serve as docking sites for signaling molecules and $\alpha$-actinin, talin and filamin belong to this subgroup; 2) potential signaling molecules form the largest group and include several kinases, such as ILK, FAK, and proteins of unknown function and 3) calcium binding and accessory molecules which probably play a role in integrin activation and the cycling or correct folding of integrins, respectively. The biological relevance of most of these interactions remains to be determined.

**Transmembrane proteins interacting with integrins**

A different class of integrin associating molecules consists of several transmembrane and membrane associated proteins, which act as co-receptors or scaffolding molecules (Table III) [104]. Members of the tetraspan protein (TM4) family or tetraspanins [105], bind directly to the extracellular domain of several integrin $\alpha$-subunits. Their interaction with integrins affects cell migration and surface expression levels of integrins, and they are thought to cluster integrins and other molecules into higher order complexes. Co-immunoprecipitation of integrins with several receptor kinases such as the EGF (epidermal growth factor)-receptor tyrosine kinase and PDGF (platelet derived growth factor)-receptor tyrosine kinase has also been reported. These assumed complexes could facilitate efficient crosstalk between adhesion and growth factor induced signaling. It has been established that both the signaling pathways induced by soluble signaling factors and integrin-ECM interactions converge at the level of FAK [106], and that integrin-mediated adhesion reduces the threshold of growth factor-mediated signaling. This cooperation of growth factor-induced and adhesion-dependent signaling is the basis for anchorage-dependent growth.
### Table II

Cytoplasmic proteins that associate with integrin cytoplasmic domains

<table>
<thead>
<tr>
<th>Protein</th>
<th>Integrin</th>
<th>Assay</th>
<th>Remarks</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Cytoskeletal proteins</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Talin</td>
<td>β: αIIb</td>
<td>P</td>
<td>cytoskeletal protein, localized in focal adhesions [147-15]</td>
</tr>
<tr>
<td>Filamin A, B</td>
<td>β1,2,3,7, P, Y2H</td>
<td>P, Y2H</td>
<td>actin cross-linking [150, 152, 153]</td>
</tr>
<tr>
<td>F-actin</td>
<td>α1, 2</td>
<td>P</td>
<td>cytoskeletal protein [154, 155]</td>
</tr>
<tr>
<td>α-Actinin</td>
<td>β1,2</td>
<td>P</td>
<td>actin cross-linking [156-158]</td>
</tr>
<tr>
<td>Myosin</td>
<td>β3</td>
<td>P</td>
<td>F-actin contraction, binds upon β3 phosphorylation [159, 160]</td>
</tr>
<tr>
<td>Skelemin</td>
<td>β1,3</td>
<td>Y2H</td>
<td>cytoskeletal/ M-band protein [161]</td>
</tr>
<tr>
<td>Plectin/HD1</td>
<td>β4</td>
<td>P, Y2H</td>
<td>binds intermediate filaments [162, 163]</td>
</tr>
<tr>
<td>p27BBP/eIF6</td>
<td>β4</td>
<td>Y2H</td>
<td>binds nuclear matrix and intermediate filaments [164]</td>
</tr>
<tr>
<td>BP230</td>
<td>β4</td>
<td>Y2H</td>
<td>binds intermediate filaments [19, 165]</td>
</tr>
<tr>
<td><strong>Adaptor and signaling proteins</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ICAP-1</td>
<td>β1</td>
<td>Y2H</td>
<td>phosphorylated upon binding, cell migration ↑ [166, 167]</td>
</tr>
<tr>
<td>Rack1</td>
<td>β1,2,5</td>
<td>Y2H</td>
<td>7x WD repeats, in vivo binding is phosphorylation dependent, binds PKC and Src [168]</td>
</tr>
<tr>
<td>Wait-1</td>
<td>β7:α4E</td>
<td>Y2H</td>
<td>5x WD repeats [169]</td>
</tr>
<tr>
<td>MBP</td>
<td>β1.A.D</td>
<td>Y2H</td>
<td>downregulated during muscle differentiation [170]</td>
</tr>
<tr>
<td>Cytohesin-1/3</td>
<td>β2</td>
<td>Y2H</td>
<td>PH and Sec7 domains with GEF activity, cell adhesion ↑ [171-173]</td>
</tr>
<tr>
<td>Mss4</td>
<td>α3</td>
<td>Y2H</td>
<td>GEF activity for Rab proteins [174]</td>
</tr>
<tr>
<td>IRS1</td>
<td>αββ3</td>
<td>P</td>
<td>contains PH domain, binds insulin receptor [175]</td>
</tr>
<tr>
<td>Paxillin</td>
<td>α4; β1,3</td>
<td>P</td>
<td>contains SH2, SH3 binding motifs, and LIM domains</td>
</tr>
<tr>
<td>Grb</td>
<td>β3</td>
<td></td>
<td>binds FAK, localized in focal adhesion [176-178]</td>
</tr>
<tr>
<td><strong>Protein kinases</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>pp125 FAK</td>
<td>β1,2,3</td>
<td>P</td>
<td>Tyr kinase, localized in focal adhesions, phosphorylated upon integrin and growth factor engagement binds Src, paxillin, Grb, p130Cas, etc [178, 183]</td>
</tr>
<tr>
<td>p59 ILK</td>
<td>β1,3</td>
<td>Y2H</td>
<td>Ser/Thr kinase contains ankyrin repeats, binds PINCH, activated by PI3-kinase, regulates GSK-3 and PKB/AKT, role in anchorage-independent growth, cell adhesion ↓ [184-186]</td>
</tr>
<tr>
<td><strong>Chaperone/Ca2+ binding</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Calnexin</td>
<td>β1; α6</td>
<td>P</td>
<td>chaperone [187]</td>
</tr>
<tr>
<td>BiP</td>
<td>αββ3</td>
<td>P</td>
<td>chaperone [188]</td>
</tr>
<tr>
<td>Calretulin</td>
<td>α</td>
<td>P</td>
<td>integrin-dependent Ca2+ influx and adhesion [189, 190]</td>
</tr>
<tr>
<td>CIB</td>
<td>αIIb</td>
<td>Y2H</td>
<td>Ca2+-dependent binding [191, 192]</td>
</tr>
<tr>
<td>Melusin</td>
<td>β1A.D</td>
<td>Y2H</td>
<td>binds in absence of cations, striated muscle specific [193]</td>
</tr>
<tr>
<td><strong>Nuclear/cytosplasmic proteins and transcription co-factors</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>β3-endonexin</td>
<td>β3</td>
<td>Y2H</td>
<td>binds cyclin A, affinity and adhesion of αIIbβ3 ↑ [194, 195, 196]</td>
</tr>
<tr>
<td>TIP20</td>
<td>β5</td>
<td>P</td>
<td>β3-endonexin-like, adhesion ↓, cell migration ↑ [197]</td>
</tr>
<tr>
<td>FHL2</td>
<td>α or β</td>
<td>Y2H</td>
<td>contains 4'': LIM domains, heart specific, localizes in focal contacts and nucleus [198]</td>
</tr>
<tr>
<td>JAB1</td>
<td>β2</td>
<td>Y2H</td>
<td>binds Jun, shuttles from plasma membrane to nucleus upon ligand binding [199]</td>
</tr>
<tr>
<td>B1N1</td>
<td>α3</td>
<td>Y2H</td>
<td>binds Myc, tumor suppressor [174]</td>
</tr>
</tbody>
</table>

P = biochemical protein-protein interaction assay; Y2H = yeast two-hybrid; SH2 = Src-homology domain 2; SH3 = Src-homology domain 3; PTB = phosphotyrosine binding domain; PH = pleckstrin-homology; GEF = guanine exchange factor
Chapter 1

Table III
Transmembrane proteins that interact with integrins

<table>
<thead>
<tr>
<th>Protein</th>
<th>Integrin</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Tetraspanins</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CD9; CD37; CD53; CD63;</td>
<td>(α3β1 and α6β1,</td>
<td>[34, 101, 104]</td>
</tr>
<tr>
<td>CD81; CD82</td>
<td>occasionally αIIb, α2β1,</td>
<td></td>
</tr>
<tr>
<td>CD151/PETA; CD151</td>
<td>α4β1, α5β1, αLβ2, α6β4</td>
<td></td>
</tr>
<tr>
<td>NAG2; CO-029</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Ig superfamily proteins</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CD147/EMMPRIN</td>
<td>α3β1, α6β1</td>
<td>[200]</td>
</tr>
<tr>
<td>CD47/IAP</td>
<td>α2β1, αvβ3, αIIbβ3</td>
<td>[201, 202]</td>
</tr>
<tr>
<td>CD46/MCP</td>
<td>α3β1</td>
<td>[203]</td>
</tr>
<tr>
<td>CD36</td>
<td>αIIbβ3, α3β1, α6β1</td>
<td>[204, 205]</td>
</tr>
<tr>
<td><strong>Growth-factor receptors</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PDGF receptor-β</td>
<td>αvβ3</td>
<td>[206]</td>
</tr>
<tr>
<td>VEGF receptor-2</td>
<td>αvβ3</td>
<td>[206]</td>
</tr>
<tr>
<td>ErbB2</td>
<td>α6β4</td>
<td>[207, 208]</td>
</tr>
<tr>
<td><strong>GPI-linked receptors</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>uPAR (CD87)</td>
<td>β1, 2, 3</td>
<td>[209-211]</td>
</tr>
<tr>
<td>FcyRIIIB (CD16B)</td>
<td>αMβ2, αDβ2</td>
<td>[212]</td>
</tr>
<tr>
<td>CD14, LPS, LBP</td>
<td>αMβ2</td>
<td>[212]</td>
</tr>
<tr>
<td><strong>Type II transmembrane proteins</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CD98</td>
<td>β1A</td>
<td>[213, 214]</td>
</tr>
<tr>
<td>BP180</td>
<td>α6β1; α6β4</td>
<td>[163, 165]</td>
</tr>
<tr>
<td><strong>Other proteins</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Caveolin</td>
<td>β1, 2</td>
<td>[211, 216]</td>
</tr>
</tbody>
</table>

Adapted from [34, 101, 104]

**Integrins in myogenesis**

The process of myogenesis includes migration of myogenic precursor cells from somites to peripheral areas, proliferation, differentiation and subsequent withdrawal from the cell cycle, and finally fusion to form mature contractile myotubes. This skeletal muscle differentiation pathway is orchestrated by myogenic basic helix-loop-helix transcription factors, including myogenin, Myf-5 and MyoD [107]. These transcription factors regulate the sequential expression of muscle specific contractile and cytoskeletal proteins that build the unique cytoskeletal structure of the sarcomere, which form the contractile building blocks of cardiac and skeletal muscle [108] (see Fig. 2.). The molecular architecture of a sarcomere facilitates the mechanical sliding of actin filaments along myosin. The polar actin filaments are mechanically linked to actin arrays of successive sarcomeres at the Z-line. The Z-line is connected at focal contact-like structures, costameres (greek for rib-like structures), to the lateral muscle fibers [109, 110]. Skeletal muscles contain adhesive structures that resemble in their protein composition the focal adhesions which are studied in cell culture; i.e.
myotendinous junctions (MTJ, muscle-tendon attachment sites). Integrins are also present in neuromuscular junctions (NMJ, axon-muscle attachment site). In contrast to the fused skeletal muscle fibers, cardiac muscle consists of mononucleated cardiomyocytes, which are connected to each other at intercalated discs. The intercalated disc consists of three classes of junctional complexes: i.e. the adherens junctions (integrin-ECM), desmosomes (cadherin-based) and gap junctions, the latter play a role in the electric coupling of heart cells.

Figure 2. Schematic representation of a skeletal and cardiac muscle cell

(A) Mononucleated myoblasts fuse to form multinucleated myofibers which differentiate into muscle fibers. Differentiated muscle cells have their cytoskeleton organized in contractile units, the sarcomeres, that facilitate the sliding of myosin (thick bars) along actin filamins (horizontal black bars). The actin filaments are linked to the sarcolemma by Z-lines (Z) at integrin rich lateral attachments sites (costameres). Specialized cell-tendon contacts, myotendinous junction (MTJ) and neuromuscular junction (NMJ) are indicated. (M = M-line).

(B) Mononucleated cardiac cells are linked together by intercalated discs, cell adhesion structures containing, integrin-based adherens-junctions, cadherin-based desmosomes and gap-junctions. The costameres are localized laterally.

Various families of adhesion molecules have been implicated in the development and biological function of skeletal and cardiac muscle. It has been demonstrated that inhibition of cell adhesion by antibodies directed against N-CAM [111, 112], N-cadherin [113, 114], ADAM12 [115] or β1 [116, 117] disturbs the differentiation and/or fusion of myoblasts into myotubes in vitro. However, most null mutations of adhesion molecules in mice appeared to have no effects on myoblast fusion, probably due to compensation by other such molecules [11, 118-120].

Studies in the fruitfly, Drosophila and the roundworm, Caenorhabditis elegans [121, 122] have indicated a role of integrins in the maintenance of muscle integrity. In Drosophila mutants, lacking the βPS integrin subunit, the architecture of the sarcomere is disrupted and the myotendinous junctions become detached after muscle contraction, despite normal myoblast differentiation and fusion [123]. Alterations of the sarcomeres have also been observed in cardiomyocytes and skeletal muscle cells, obtained after in vitro differentiation of embryonic stem cells, lacking β1 [124, 125].
Fetal myoblasts express α5β1, α6β1 and αvβ3 integrins, which are down regulated during myogenesis [126-129]. These integrins have been suggested to play a role in myoblast migration and myofibrillogenesis. The relative ratios in expression of these integrins was found to influence myogenesis in vitro [130, 131]. However, the absence of the α6 integrin subunit does not lead to detectable cardiac or muscular defects [58], although α5 deficiency, as analyzed in chimeric mice, causes muscular dystrophy [132]. A transient expression of α4 has been implicated in the secondary wave of myoblast fusion [133]. However, loss of α4 in experiments with α4 null chimeric mice and in in vitro myogenesis studies of α4-deficient ES cells, showed no obvious muscular defects [134]. During myogenesis expression of the α7β1A integrin is first induced. Subsequently, α7β1A is replaced by the α7β1D and α7α1D integrins, which are the major integrin variants in adult muscle (this thesis, [135]). Expression of the collagen receptors α10β1 and α11β1 in adult skeletal muscle tissue has also been reported [129, 136-138], as has the induced expression of the integrin α9β1 [139, 140]. Notably, expression of the α1β1 and α3β1 integrins in myoblasts has only been detected in in vitro myogenesis studies [141, 142].

Cardiac cells express different integrins during heart development. Integrins α1β1, α3α1 and α6α1 are expressed at low levels at the onset of cardiogenesis [143]. Subsequently, the expression of α1β1 and α3α1 decreases, while α6α1 remain present [144, 145]. In addition, the integrin α7β1 is induced in heart postnatally where it remains the only variant of α7 expressed in adult heart [146].

Scope of this thesis

In this thesis studies on the expression and the role of a splice variant of the cytoplasmic tail of the β1 integrin subunit are presented. In chapter 2 the cloning of the integrin β1D cytoplasmic variant is described, which is specific for skeletal and cardiac muscle. Chapter 3 investigates the expression pattern of β1D integrins in adult muscles and during mouse embryogenesis. It is shown that the expression of β1D is specific for adult skeletal and cardiac muscle cells, where it is localized at muscle specific cell-matrix junctions: the costameres, neuromuscular- and myotendinous junctions in skeletal muscle, and the intercalated disks and costameres in cardiac muscle. Furthermore, the expression of the ubiquitous β1A is switched towards β1D in muscle tissues during mouse embryonic development. In chapter 4 the effects of the expression of either of the splice variants β1A or β1D in, a β1-deficient epithelial cell line, GE11 are outlined. Expression of either of the β1-splice variants induced the disruption of intercellular adhesions and cell scattering. The functional relationship between integrins, cadherins and Rho-like GTPases was further analyzed. In chapter 5 the filamin protein family of actin cross-linking proteins is reviewed. In chapter 6 we show that a novel splice variant of filamin-B interacts with the cytoplasmic domains of both β1A and β1D. Cell transfection studies with Green fluorescent protein-tagged full length filamin-B variants indicated that filamin-B isoforms have different cellular localization and different effects on myogenesis. In chapter 7 a summary of and a discussion on the role β1 integrin variants in myogenesis and signaling is presented.
References

Chapter 1

[45] M.S. Bretscher, Circulating integrins: α5β1, α6β4 and Mac-1, but not α3β1, α4β1 or LFA-1, EMBO. J. 11 (1992) 405-410.


[75] A. Kääpä, K. Peter and J. Ylänne, Effects of mutations in the cytoplasmic domain of integrin β1 to talin binding
Chapter 1


[128] K.A. Mcdonald, M. Lakonishok and A.F. Horwitz, αv and α3 integrin subunits are associated with myofibrils


[149] I. Knezevic, T.M. Leisner and S.C.T. Lam, Direct binding of the platelet integrin αIIbβ3 (GPⅡb-Ⅲa) to talin. Evidence that interaction is mediated through the cytoplasmic domains of both αIIb and β3, J. Biol. Chem. 271 (1996) 16416-16421.


F.M. Pavalko and S.M. LaRoche, Activation of human neutrophils induces an interaction between integrin β2-subunit (CD18) and the actin binding protein α-actinin, J. Immun. 151 (1993) 3795-1807.


Z. Hmama, K.L. Knutson, P. Herrera-Velit, D. Nandan and N.E. Reiner, Monocyte adherence induced by
Chapter 1


[197] S. Tang, Y. Gao and J.A. Ware, Enhancement of endothelial cell migration and in vitro tube formation by
General introduction


