Biochimica et Biophysica Acta 1788 (2009) 779–789

Contents lists available at ScienceDirect

Biochimica et Biophysica Acta

journal homepage: www.elsevier.com/locate/bbamem

Review

Integrins during evolution: Evolutionary trees and model organisms

Mark S. Johnson [a], Ning Lu [b], Konstantin Denessiouk [a], Jyrki Heino [c], Donald Gullberg [b,*]

[a] Department of Biochemistry and Pharmacy, Åbo Academy University, Turku, Finland
[b] Department of Biomedicine, University of Bergen, Bergen, Norway
[c] Department of Biochemistry, University of Turku, Turku, Finland

ARTICLE INFO

Article history:
Received 13 March 2008
Received in revised form 3 December 2008
Accepted 23 December 2008
Available online 7 January 2009

Keywords:
Integrin
Evolutionary tree
Model organism
Muscle formation
Human
Chordate
Invertebrate
Metazoan

ABSTRACT

The integrins form a large family of cell adhesion receptors. All multicellular animals express integrins, indicating that the family evolved relatively early in the history of metazoans, and homologous sequences of the component domains of integrin α and β subunits are seen in prokaryotes. Some integrins, however, seem to be much younger. For example, the αI domain containing integrins, including collagen receptors and leukocyte integrins, have been found in chordates only. Here, we will discuss what conclusions can be drawn about integrin function by studying the evolutionary conservation of integrins. We will also look at how studying integrins in organisms such as the fruit fly and mouse has helped our understanding of integrin evolution–function relationships. As an illustration of this, we will summarize the current understanding of integrin involvement in skeletal muscle formation.

Contents

1. Introduction

1.1. Cell adhesion during evolution and development

Cell adhesive mechanisms act during development to regulate tissue formation, and multicellular organisms have developed increasingly refined mechanisms for regulating cell adhesive events in the course of their evolution. Interactions with the extracellular matrix (ECM) via cell surface receptors have been shown to be

* Corresponding author. Jonas Lies vei 91, N-5009 Bergen, Norway. Tel.:+47 55586332; fax:+47 5558641.
E-mail address: donald.gullberg@biomed.uib.no (D. Gullberg).

0005-2736/$ – see front matter © 2009 Elsevier B.V. All rights reserved.
doi:10.1016/j.bbamem.2008.12.013

important during several steps in embryonic development. Cell–cell contacts need to be formed and broken in a dynamic manner during the early steps in organogenesis, and cell migration events are needed to direct cells to the right places during remodelling and growth phases. Towards the end of organogenesis, a complete set of static interactions are needed to keep the cells in place in the newly formed tissues. Most importantly, another set of contacts is then required to allow for postnatal growth, being finally replaced by stable contacts that keep the cells in place in the adult tissues.

The demands on adhesive events of the type that need to be regulated also increase with the formation of new physiological systems, such as a closed circulatory system or a complete immune system [1,2], in both of which cells circulating in the body constantly need to be ready to engage in transient interactions such as in immune surveillance, blood clotting and inflammation. The need to change and adapt cell adhesive interactions must also be retained in readiness for regeneration events following tissue damage, often involving the recruitment of stem cells of various types.

1.2. Integrins

Integrins have many roles during development. Since all nucleated cells in the body have a specific repertoire of integrins, these roles are complex and are integrated with the specific functions of the cells in different tissues.

The functions of integrins at the cellular level can be grouped into those related to their roles as mechanical links in cell adhesion and migration, and their signalling function [3]. The integrin-dependent effect on total signalling in a certain cell type can influence cytoskeletal arrangements, transcriptional activity and a variety of metabolic reactions. These roles then become even more complex in a tissue context, as illustrated by findings that integrins can regulate the activity of paracrine loops of secreted growth factors [4–6].

When trying to understand the role of integrins in developmental processes, two types of insight can be gained from studying model organisms located at different positions in an evolutionary tree: a detailed understanding of a particular integrin orthologue in another species, or general knowledge about how integrins influence a developmental process, knowledge which is not necessarily tied to a specific integrin.

Examples of the latter include the general importance of integrins for skeletal muscle integrity in *Drosophila* and mammals. Recent fruit fly data show that integrins are important for setting up the stem cell niche [7,8], and it is an exciting possibility that the findings made regarding the *Drosophila* testis might also have implications for future stem cell-based therapeutic approaches in humans.

In this review we will discuss what can be learned about integrin structure and function from comparisons of integrins between species. We will illustrate the usefulness of studying different model organisms in order to understand integrin function in a specific tissue by comparing skeletal muscle development in the fruit fly (*Drosophila melanogaster*), and mouse (*Mus musculus*). Several excellent reviews on the role of integrins in *Caenorhabditis elegans* exist [3,9], but in the current review we have chosen not to discuss this nematode. Some additional animal models will be mentioned in relation to their potential as model organisms for future use.

2. Integrins during evolution

2.1. General

Integrins are heterodimers composed of one α and one β subunit. Present-day mammals are known to express 18 different integrin α subunits and 8 β subunits [10]. The model of integrin synthesis indicates that the promiscuous subunits, such as β1 or αV, are produced in large excess when compared to the other subunits [11,12]. In the endoplasmic reticulum integrin subunits find their binding partners and form heterodimers. However, only 24 different αβ combinations are observed in human (Table 1) and the monomeric integrins never reach the cell surface. The integrin α and β subunits have separate evolutionary histories, but the evolution of new heterodimers may have required concomitant changes in both the α and β subunits.

The α and β subunits in human have clearly recognizable homologues in organisms that diverged from the tree of life long before the appearance of the first vertebrates. Indeed, the origins of the subunits or component pieces appear early in evolution and predate the chordate line and in the case of von Willebrand factor A (vWFA) they are found in prokaryotes [13,14]. We have also seen that large amino-terminal regions of both the α and β subunits that respectively code for the β propeller and the βI domain in the integrins are found in protein sequences from bacteria (see below).

The general function of the integrins – a role in cell adhesion and communication – appears to have been established early as well and

Table 1
Mammalian integrins.

Integrin	Ligands	Location of defects in knock-outs and/or main expression sites
Human integrins containing a PS1 group α subunit		
α3β1	Laminins (collagens)	Skin, kidney, lung, cortex
α6β1	Laminins, ADAMs	Gametes, macrophages, platelets
α6β4	Laminins	Skin (hemidesmosomes)
α7β1	Laminins	Muscle
Human integrins containing a PS2 group α subunit		
α5β1	Fibronectin (RGD)	Embryonic development (blood vessels)
α8β1	Fibronectin, vitronectin, tenascin C, osteopontin, nefronectin (RGD)	Kidney, inner ear
αVβ1	Fibronectin, vitronectin (RGD)	Not clear, whether expressed *in vivo*
αVβ3	Fibrinogen, fibronectin, vitronectin, tenascin C, osteopontin, bone sialoprotein (RGD), MMP-2	Bone (osteoclasts)
αVβ5	Vitronectin (RGD)	Eye (retinal phagocytosis), bone (osteoclastogenesis)
αVβ6	Fibronectin, TGF-β-LAP (RGD)	Skin, lung (collagen accumulation)
αVβ8	Vitronectin (RGD)	Vascular development
αIIbβ3	Fibrinogen (RGD, GAKQAGDV), Fibronectin, vitronectin (RGD)	Platelets
Human integrins containing an α4/α9 group α subunit		
α4β1	Fibronectin, VCAM	Embryonic development (heart)
α4β7	Fibronectin, VCAM, MadCaM	Peyer's patch (immune system)
α9β1	Tenascin C, osteopontin, ADAMs, factor XIII, VCAM, VEGF-C, VEGF-D	Lymphangiogenesis
Human integrins containing an α subunit with an αI domain		
Collagen receptor subgroup		
α1β1	Collagens, semaphorin 7A, (laminins)	Mesenchymal tissues
α2β1	Collagens, tenascin C, (laminins)	Platelets, epithelium, mast-cells (mesenchymal tissues)
α10β1	Collagens	Cartilage
α11β1	Collagens	Periodontal ligament
Leukocyte integrin subgroup		
αDβ2	ICAM, VCAM	Eosinophils
αMβ2	ICAM, VCAM, iC3b, factor X, fibrinogen	Leukocytes (phagocytosis)
αLβ2	ICAM	Leukocytes (recruitment)
αXβ2	Fibrinogen, plasminogen, heparin, iC3b	Leukocytes
αEβ7	E-cadherin	Skin, Gut (immune system)

The heterodimers have been organized based on the phylogenic subgroup of the α subunit.

has been well characterized in several invertebrates (for a review, see [15]) such as in *C. elegans* where two α subunits (Ina-1, Pat-2) and one β subunit (Pat-3) [16–18] are found and five α and two β subunits in *Drosophila* [19–25].

In vertebrates, the integrin subunits have diversified to accommodate additional functions with roles that likely relate to the large changes that occurred during chordate evolution in the transition to cartilaginous and bony internal structures, a dual innate and adaptive immune system and the high-pressure circulatory system that characterize the vertebrate lineage. Thus, the vertebrate integrin subunits did not appear suddenly, but arose from homologous sequences and domains that preexisted their functions in cell adhesion in the first metazoans and existing within the protists and prokaryotes.

2.2. Integrin α and β subunits

The mammalian α subunits can be divided into four different main groups but do not have representatives within a fifth cluster comprising sequences from insects (Fig. 1) [13,26–29]. One subfamily of integrin α subunits (α3, α7, α6) is evolutionarily related to *Drosophila* PS1 proteins (Table 1). Sequences from invertebrates, including *C. elegans* (Ina-1), *Drosophila* (PS1), and the urochordates *Polyandrocarpa misakiensis* and *Ciona intestinalis* (tunicate; ascidian; sea squirt), cluster as outliers within this grouping indicating that they are ancestral to but not direct functional orthologues of the present-day mammalian subunits. *Drosophila* PS1 and the mammalian orthologues have all evolved to recognize members of the laminin family [30]. Laminins are important structural proteins in basement membranes. Mammalian PS1 group α subunits can form heterodimers with β1 or in one case with β4 subunits. These integrins are important for the integrity of tissues, including kidney, skin and muscle.

Another subgroup of integrin α subunits, namely αIIb, αV, α5, and α8, is structurally related to the *Drosophila* PS2 protein (Table 1). As with the PS1 cluster, the known invertebrate sequences cluster as ancestral homologues but not direct orthologues of the mammalian sequences (asterisks in Fig. 1 indicate the relative branch points for invertebrate sequences on the generalized tree for the vertebrate integrin α subunits). Other invertebrate sequences include those of *C. elegans* (Pat-2), the echinoderms *Strogylocentrotus purpuratus* and *Lytechinus variegates* (sea urchins), and the urochordates *Halocynthia roretzi* and *C. intestinalis*. The α integrin from a very early metazoan, the sponge *Geodia cydonium* [31], is also reported to cluster near the PS2 subgroup [27].

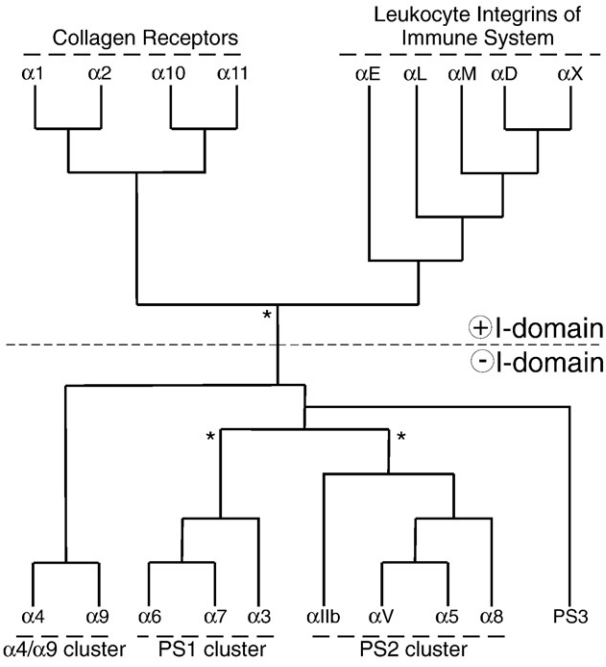

Fig. 1. Generalized tree for the integrin α subunits present in human that are most probably found within the entire vertebrate lineage. The PS3 cluster only contains invertebrate sequences but some invertebrate sequences also cluster as outliers of the vertebrate clusters (asterisks indicate the branch point). The earliest diverging α subunits containing an I domain appear in the tunicates as outliers to both the collagen receptor and leukocyte clusters. Branch lengths are arbitrary.

The receptors of the PS2 group have evolved to recognize a short motif, such as the classic RGD sequence, in extracellular matrix (ECM) proteins. In mammals, there are several RGD-containing ECM proteins, such as fibronectin. *Drosophila* has no orthologue for fibronectin, but PS2 recognizes other ECM proteins, such as tiggrin [32]. In mouse, one of the RGD-dependent mammalian integrins, that is α5, seems to be indispensable for early embryonic development [33], stressing the fundamental nature of RGD-dependent cell adhesion and locomotion. *G. cydonium* contains a putative multiadhesive protein possibly containing a fibronectin FN_3 module, a sequence (KILDA) similar to EILDV that is recognized by some mammalian integrins, but no RGD motif [34].

Other mammalian integrins in the PS2 group have much more specific functions in bone cells, kidney cells, vascular development etc. (Table 1). Interestingly, in vertebrates the RGD motif is also found in non-ECM proteins. Cell adhesion to plasma fibrinogen is integrin-mediated and RGD-dependent. In addition to the RGD motif αIIβ3 integrin on platelets can recognize another similar sequence in fibrinogen, namely GAKQADV [35]. Furthermore, the activation of a growth factor, namely the latent form of transforming growth factor β (TGF-β), may be mediated by integrins that recognize a RGD motif in the LAP (latency associated peptide) domain of latent TGF-β [36]. These examples suggest that integrins have also received new functions due to evolution in other protein families.

While the PS1 and PS2 group integrins have been found in many invertebrates, including *C. elegans*, a third subgroup of *Drosophila* integrin α subunits, named PS3, with representatives also seen in the butterfly *Pseudoplusia includens* [28], seems to be specific for insects. Two mammalian α subunits, namely α4 and α9 can be considered as a separate subgroup (Table 1). These integrins recognize, in addition to ECM proteins, certain plasma proteins, counter receptors belonging to the immunoglobulin superfamily and vascular endothelial cell growth factors [37].

A structurally and functionally unique subgroup of integrin α subunits is formed by integrins that contain a specific "inserted" or "αI" domain. Because of the structural similarity to the von Willebrand factor "A" domain these domains are also often called integrin "αA" domains. αI domains form the ligand recognition part of the corresponding integrins. In mammals, nine out of 18 α subunits have the αI domain. Four αI domain integrins are collagen receptors (reviewed in: [38,39]), while the other five are expressed on leukocytes (reviewed in [40]) and they recognize counter receptors, such as E-cadherins or intercellular and vascular adhesion molecules (ICAMs or VCAMs, correspondingly). Some of them also bind to plasma proteins such as fibrinogen or component iC3b in the complement system (Table 1). Similarly the collagen receptor subgroup of integrins have at least a limited ability to bind to other ECM proteins including the laminins. Semaphorin 7A participates in the regulation of axon guidance and activation of inflammatory cells and was recently described to be recognized by the α1β1 collagen receptor [41].

Interestingly, the αI domain integrins have not been found in invertebrates, such as insects and worms. Recently, up to eight αI domain integrin genes were recognized in the genome of *C. intestinalis*, representing the most primitive chordates [26,28,29,42]. The *Ciona* αI domain integrins form a separate group of αI domain integrins that is different when compared to either mammalian

collagen receptors or leukocyte integrins [26,28]. The functional testing of *Ciona* integrin αI domains has indicated that they cannot recognize triple helical, GFOGER (O=hydroxyproline) sequence containing peptides [42] in contrast to all vertebrate collagen receptor αI domains that can [43,44]. At the moment it is unclear what the actual function of these integrins is. However, the single αI domain integrin found in another ascidian species, *H. roretzi*, is reported to act as a complement receptor and participate in phagocytosis by hemocytes [45,46], but the sequence does not cluster as a direct orthologue of the mammalian type integrin I domains [13].

A sequence-based search revealed that an amino-terminal region from integrin α subunits corresponding to the β propeller domain is recognizable in several bacterial sequences (unpublished). For example in a sequence from *Nitrosococcus oceani* (accession code YP344083; [47]). This similarity is probably why the sequence was automatically labeled as "Integrin alpha chain" even though the sequence similarities appear to be limited to the β propeller region of integrin chains.

The integrin β subunits seem to form three major phylogenetic branches (Fig. 2), with those present in vertebrates termed groups A (β1, β2 and β7) and B (β3–β6 and β8) and a third branch containing only invertebrate sequences [28]; this segregation agrees well with the earlier phylogenetic classification by Hughes [27] and the branching orders that have been reported [13,26–29]. Ewan et al. [26] have considered the β4 branch to be a separate group of one, thus segregating the β subunits into Beta 1–3 plus a Beta ascidian group. In our three-branch classification, branch A contains β subunits that in human are associated with most of the alpha subunits and from the PS1, PS2 and I domain clusters: promiscuous β1 associates with α1–α11 and αV; β2 with αD, αL, αM, and αX; and β7 with α4 and αE. Branch B contains the β3, β5, β6 and β8 subunits that can each form a heterodimer with αV (PS2), β3 also forms heterodimer with αIIb (PS2), whereas β4 from branch B forms a heterodimer with α6 (PS1). A *Ciona* β subunit clusters with the β4 integrins and as outliers to the cluster containing the β1, β2 and β7 subunits (asterisks in Fig. 2), whereas multiple β subunits from *Ciona* and *Halocynthia* are part of the invertebrate group [26,28,29]. In addition to the α integrin from the sponge *G. cydonium* [31], a β subunit from this sponge has also been sequenced [48], as well as β subunits from other Porifera including the coral *Acropora millepora* and sponge *O. tenuis*. Furthermore, sequences homologous to domains from integrin β subunits extend into the prokaryotes. For example, a sequence from the cyanobacterium *Trichodesmium erythraeum* has been automatically classified as a "β-like integrin" (accession code YP 721619). Our own alignment of the sponge and cyanobacterium sequences with the mammalian sequences clearly shows that the cyanobacterium sequence is homologous with a large portion of the first ~450 amino acids, which would correspond to the amino-terminal βA-domain and part of the hybrid domain of the vertebrate integrin β subunits. However, the EGF repeats apparently are not present in the *T. erythraeum* sequence as evidenced by the lack of repetitive conserved cysteine residues (unpublished).

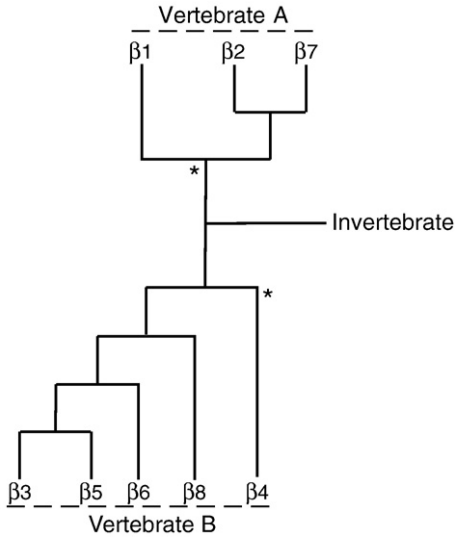

Fig. 2. Generalized tree for the integrin β subunits present in human that are most probably found within the entire vertebrate lineage. Invertebrate sequence primarily cluster as a single group but tunicate sequences are seen to cluster as an outlier of the A group as a whole and as an outlier of the (4 integrins of the B group of vertebrate sequences. Branch lengths are arbitrary.

Three-dimensional structures of the ectodomain of human αVβ3 [49] and αIIbβ3 [50] have defined the structure of the individual domains and suggested that the integrin heterodimer undergoes large conformational changes related to outside-in and inside-out signalling events [49,51,52]. The sequence similarities and conservation of these domains in other species suggest that the overall structures have remained the same over evolutionary time and that the dynamic nature of the mammalian integrins may have been established already in the earliest recognizable integrins of the prokaryotes and the earliest metazoans. The structure of αVβ3 in complex with an RGD peptide [53] has pinpointed the binding site for this motif present on many proteins of the ECM; transmission electron microscopy has shown that this bent conformation of the ectodomain can also bind a fibronectin FN_3 fragment containing multiple domains [54]. No X-ray structure has yet been obtained for an αI domain-containing integrin although quite reasonable models have been estimated based on the known X-ray structures of an I domain and the αVβ3 ectodomain [55]. Nishida et al. [56] have used electron microscopy to visualize the location of the I domain relative to the rest of the headpiece of both the αXβ2 and αLβ2 integrins in various conformations from bent to extended.

2.3. Origin of the vertebrate integrins

The available sequence data demonstrate that the integrins have a very early history. Both α and β subunits have been found throughout the invertebrates, including one of the earliest metazoans, a sponge *G. cydonium*. Not only does the presence of integrin subunits appear to extend right through the invertebrates to the earliest metazoans, but large portions of the sequences from the α and β subunits are detectible in prokaryotic sequences. Domains of the vWFA family have been used for a wide variety of functions throughout evolutionary history [13,14,57], not only in multicellular organisms but in unicellular eukaryotes and prokaryotes, too. Surprisingly, I-domain containing α integrins are a much more recent phenomena (see below).

Although the picture is far from complete and limited by the genomes that have been sequenced, assembled and annotated, we do know that orthologues of the mammalian integrin subunits are present in the bony fish, where duplicate forms of both subunits have been observed (e.g., multiple β1 orthologues in zebrafish *Danio rerio*, and duplicates of α5, α6, α10 and α11 in the pufferfish, *Takifugu rubripes*) [28,58], but orthologues of mammalian integrin subunits have not been detected in the tunicates whose genomes have been sequenced (e.g. *Ciona*). Furthermore, orthologues have not been reported for any of the organisms whose divergence precedes the chordate evolutionary line, whereas ancestral sequences are observed in tunicates as well as in other invertebrates that are clearly homologues of the α and β integrins. As a result, we can bracket the origin of the present day vertebrate set as having diverged from the early integrin forms in species coming after the divergence of the tunicates and certainly before the divergence of the bony fish.

There are several aspects that make it difficult to pinpoint the moment in the history of life when vertebrate integrin forms were

first established. Firstly, sequence data is currently lacking for species that diverged after the tunicates and before the bony fish. Secondly, several mass extinctions [59] especially during the Paleozoic era may have reduced the number of present data representatives quite considerably. Extant forms include amphioxus (a lancelet; Cephalochordata); the jawless vertebrates that include various species of lamprey and hagfish (Agnatha); and the Chondrichthyes (Elasmobranchii — sharks and rays, and the Holocephalii — the chimaeras, (ratfish)). Nonetheless, the extant group of chordates representing the earliest divergence along the chordate line and containing authentic orthologues of the mammalian integrins should be established in the near future. Currently, the genomes of several species within Chondrichthyes are being sequenced: *Callorhinchus milii* (elephant shark or ghost shark), *Ginglymostoma cirratum* (nurse shark), *Squalus acanthias* (spiny dogfish) and the *Raja erinacea* (little skate). Given the large number of vertebrate features shared by sharks and rays in comparison with bony fish and the tetrapods, it would be surprising if orthologues of the human-type α and β subunits were not eventually reported. Several genome-sequencing projects are also in progress on some intervening species between the Urochordates and Chondrichthyes: i.e., the cephalochordate *Branchiostoma floridae*, the Florida lancelet, and *Petromyzon marinus*, the sea lamprey.

The absence of any report of an αI domain containing integrin in non-chordate invertebrates suggests that these integrins have clearly evolved quite late in the history of the metazoans. Based on their functional testing it can be speculated they have been recruited during the development of the immune system. Mammalian leukocyte integrins are essential for the immunity and some members in the collagen receptor subgroup of the αI domain integrins also mediate leukocyte adhesion to collagens [38]. Furthermore, the mammalian α2 subunit has been linked to innate immunity [60].

The collagens themselves form a very large family of ECM proteins, which has been considered to play an essential role in the evolution of metazoans since their earliest origins [61–64]. Furthermore, collagens are among the most abundant proteins in tissues. Therefore, it is quite intriguing that the integrin-mediated mechanisms of direct collagen binding have not evolved earlier. The late appearance of these receptors may also be seen in the fact that the lack of collagen receptor integrins has very little effect on mouse development [38,39]. In addition to immunological functions, the collagen receptors seem to participate in the homeostasis of connective tissues [38,39]. The three-dimensional structures of vWFA domains [65–67]and αI domains (αL [68], αM [69], α1 [70], α2 [71]) solved using X-ray crystallography have revealed their close structural similarity and location of the ligand binding metal ion binding MIDAS site. The α subunit ligands bind to MIDAS of this domain, and the domain itself has been inserted within a repeat of the amino-terminal β-propeller domain. The complex of α2I with a collagen-like triple helix has defined the structural changes that occur in the collagen binding integrins, where a local conformational change involving helix C and shifting the domain into the "open" form is key to ligand recognition [55]. The presence of this helix defines the integrins of the collagen-binding subset: α1, α2, α10 and α11.

2.4. What has driven the evolution of the integrins?

There are obvious large-scale developments that were essential to the development, survival and success of multicellular organisms: cell–cell interactions, cellular communication and development of large-scale supportive structures (i.e., cartilage and bone in the vertebrates) and the necessity to remodel a multicellular organism during the various stages of the life cycle; the development of the early innate immune response and rise of the adaptive immune response; and the development of a low-pressure circulatory system that is later replaced by the high-pressure circulatory system. Proteins of the ECM are key to many of the interactions that take place and various proteins of the ECM have been established with the earliest metazoans.

The integrins have an early history that even predates their function in metazoan cell–cell communication. This is not unusual, since genes do not appear *de novo* when a new function at the protein level is taken on, but instead arise from preexisting genes via gene duplication, mutation, and combination and fusion of functional domains. In the case of the integrins, large changes in the organization of cells in multicellular organisms have gone hand-in-hand with changes in integrin subunit diversity, and a large increase of integrin subunits types appear in the vertebrates. Although some collagens have been present very early in metazoan evolution, recognition of collagens by integrins has appeared only relatively recently in one subset of the α subunits that contain I domains. In contrast, the cell-adhesion system via the RGD motif appears to have a very early origin, possibly functioning in cell-substratum attachment in the unicellular protozoan *Neoparamoeba aestuarina* [72]. We have seen that representative sequences of both α and β integrin subunits are found in bacteria but that their functions in these single-cell organisms are unknown at this time. With regard to the identification of the earliest appearance of true orthologues of the vertebrate subunits represented in extant chordates, the current genome sequencing projects should clarify this question within the very near future.

3. Studies of integrin function in the skeletal muscle of model organisms

3.1. Introduction

Cell adhesion is a fundamental process during early embryonic events such as gastrulation, somitogenesis and myogenesis. For each of these morphogenetic processes cell adhesion must be closely regulated. With chordates and vertebrates new organ systems are introduced and most protein families diversify to become subspecialized as discussed in Section 2. In higher vertebrates, identifying the function of a large gene family in a particular tissue can be difficult, and here model organisms with smaller genomes such as *Drosophila* offer simpler alternatives to study principles of molecular mechanisms. Whereas many aspects of myogenesis have changed during evolution, some fundamental cell adhesive events have remained surprisingly intact. As some cells in vertebrate muscle tissues needed to interact with fibrillar collagens, the integrins on these cells have evolved to include new domains that enable these interactions. Muscle offers a fascinating tale of how molecular principles have evolved in tissues and have been conserved, but also of how protein families have subspecialized in order to engage in new functions. Below we will summarize some current state-of-the art knowledge pertaining to muscle cell adhesion, focusing on fruit fly and mouse.

3.2. D. melanogaster

The fruit fly goes through a number of developmental stages such as egg, larva and pupa before the emergence of a mature insect. The relative ease with which one can obtain mutants and the availability of tools for analysing genetic interactions have made *Drosophila* a favourite model organism among biologists. Another major advantage is the smaller size of the genome, with correspondingly smaller gene families, thus causing less redundancy problems when analysing gene function. Studies of integrin function in *Drosophila* have focused on the formation of the midgut, muscle and wing [9,73], but some elegant work on axon guidance [74,75] and germ stem cells has also been performed more recently [7,8]. Recent data on the role of integrins during the establishment of a stem cell niche in the *Drosophila* testis [7,8] illustrate why clinicians involved with stem cell therapy should keep an eye on the fly literature. These data indicate that integrins play an important role in the correct positioning of the stem cell niche

within the tissue. If this is a conserved biological principle, it might have consequences for stem cell therapy and the use of artificial carrier materials. Depending on the source of the stem cells, one can thus envisage how stem cells might in the future have to be equipped with certain integrins in order to position themselves at the right place within the tissue.

As mentioned in Section 2 the integrin family in *Drosophila* is composed of 7 subunits, 5 α and two β subunits [76]. Some information on the integrin chains, which we will discuss in the context of muscle formation, i.e. αPS1, αPS2 and βPS3, is shown in Table 2.

In *Drosophila*, the formation of the embryonic somatic body muscles, which are later used mainly by the larva for crawling, involves MyoD-positive founder cells that prefigure the final muscle pattern and fusion of these with a second population of fusion-competent myoblasts [77–79]. The muscle cells then go through various stages in which they form contacts with specialized cells called tendon cells, which are connected to the cuticular exoskeleton. During this process the muscle cells extend and send out filipodial sensing extensions to make contact with the tendon cells [80]. In the absence of βPS integrins, cell fusion is defective, the rudimentary muscles show defective cytoskeletal attachments and the muscle cells come lose from their attachment points midway through embryogenesis [21,81–83]. Severe defects were also observed when integrin-deficient myoblasts were isolated and cultured *in vitro* [84].

Some molecular details of muscle attachment have remained obscure until recently. The muscle cells on the muscle side of the junction express the αPS2 integrin chain, whereas the αPS1 integrin subunit is expressed on the adjoining tendon cell side [85], and there is a basement membrane containing the integrin ligands tiggrin [32], laminin α5β1γ1 [86] and laminin α2β1γ1 [87] in between the cell layers. *Drosophila* laminin with the composition α5β1γ1 was the first αPS1βPS ligand to be identified [30], while the RGD-containing proteins tiggrin [32] and laminin with the composition α2β1γ1 were identified as αPS2βPS ligands in later *in vitro* studies [88]. Surprisingly, the incorporation of tiggrin into the muscle matrix *in vivo* is largely normal in *mys* mutants [32], and absence of the laminin α5 chain likewise has only relatively minor effects on somatic muscle attachments. On the other hand, the laminin α2 chain appears to be crucial for proper muscle attachments to form [88,89]. Thus, although tiggrin and laminin α5 β1γ1 were identified as integrin ligands, which are both present in the muscle junction, genetic data indicate that they play a relatively minor role at the muscle attachment site *in vivo*. The nature of the alternative αPS1βPS and αPS2βPS ligands has also remained elusive, and new, unexpected findings have emerged only recently. Firstly, it turns out that a protein synthesized in the tendon cells, *Drosophila* thrombospondin, depends on αPS2 for its organization into the muscle matrix, and is thus one major αPS2βPS ligand *in vivo* [90,91]. Secondly, the transmembrane muscle cell protein perdido, which curiously enough contains a laminin-like motif, appears to act as a ligand for αPS1βPS on tendon cells [92]. To add further complexity to this picture, perdido interacts with the intracellular proteins Grip, which in turn might allow for *cis* interactions with the muscle integrin αPS2βPS in the muscle plasma membrane. These data thus show that the muscle–tendon cell linkage in *Drosophila* muscle at the molecular level is more intimate than was previously thought and involves an unexpected direct cell–cell mode of interaction (Fig. 3). The relationship between the muscle cell ligands laminin α2β1γ1 and thrombospondin is currently unclear, however. Why do muscle integrins need two high-affinity ligands? It is possible that they could have different functions at different stages in muscle formation. The finding that some laminin mutations seem preferentially to affect the attachment of certain muscles [89] indicates that the relative contribution of different attachment mechanisms varies between muscle groups. Recently, a similar heterogeneity in laminin isoform expression has been shown in mouse extraocular muscle [93].

Table 2
Drosophila integrin chains involved in muscle formation.

Drosophila integrin chain	Mutant	Phenotype	Extracellular ligands	Vertebrate orthologue(s)
βPS3	*myospheroid (mys)*	Embryonic lethal, muscle detachment	n.a.	β1
αPS1	*multiple edematous wings (mew)*	Larval lethal, midgut defect	Perdido, laminin α5β1γ1	α3, α6, α7
αPS2	*inflated (if)*	Embryonic lethal, muscle detachment	Thrombospondin, tiggrin, laminin α2β1γ1	α5, αv, α8

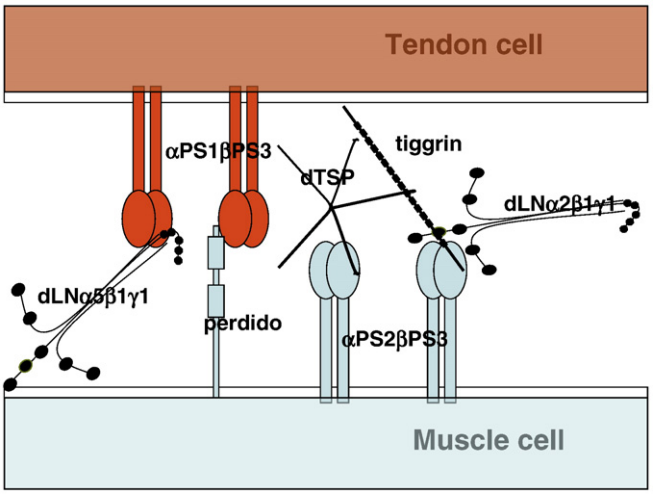

Fig. 3. Schematic illustration of integrin–ligand interactions at the muscle–tendon cell junction in *Drosophila*. Binding of perdido to the integrin established a cell–cell contact. It is unclear how the ECM network is linked to the tendon side of this junction. dTSP; *Drosophila* thrombospondin.

One lesson from the *Drosophila* system is thus that integrins in skeletal muscle serve as an important mechanical linkage at muscle cell junctions linking muscle cells to the basement membrane. Furthermore, *in vitro* studies of cultured embryonic *Drosophila* muscle cells demonstrated a role for integrins in myoblast fusion and sarcomere stability [84,94]. The molecular basis for these integrin-dependent effects on the cytoskeleton and differentiation is still not understood. The findings that soluble integrin ligands promote sarcomere formation might reflect activation of integrin signalling, indirectly affecting sarcomere formation. Whereas the integrin ligands in the *Drosophila* muscle junctions have only recently been clarified, the available knowledge about the ligands in mouse muscle is in some aspects more rudimentary (see below).

3.3. M. musculus

All the integrin genes have now been inactivated in mice [114–116], but our understanding of integrin function in mouse development is far from complete. In the case of embryonic or postnatal lethal phenotypes, the death of the animals precludes any detailed analysis of integrin function. We can expect to learn more about integrin function from conditional knockout animals in the next 10 years.

Some data have already started to emerge from detailed conditional knockout studies, however [117], although it should be remembered that knockout mice are not always the ideal animals when creating disease models and attempting close comparisons with the corresponding human condition. One striking example of this concerns dystrophin mutations, which are well tolerated in mice, whereas they cause a fatal condition in humans [118]. Another example illustrating the complexity of animal models is the recent

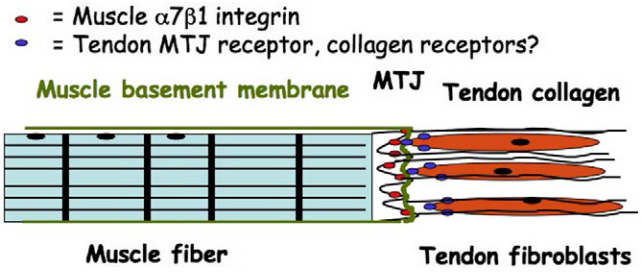

Fig. 4. Schematic illustration of the myotendinous junction in the mouse. Muscle fibres are attached to the terminal basement membrane at the myotendinous junction (MTJ) via α7β1 integrin. It is unclear how the fibroblasts are attached at the myotendinous junction.

work on cherubism in mice. A knockout model showed no phenotype in the bone of mice deficient in the adaptor protein SH3BP2, but transgenic animals with a disease mutation in the gene encoding this protein yielded a dramatic phenotype [119,120]. Great care must thus be taken when using a model organism to create disease models.

Based on the early findings in *Drosophila*, it was postulated that integrins would have similar functions in vertebrate muscle cells, i.e. affecting myoblast fusion and sarcomere stability [84]. Early experiments utilizing β1 integrin knockout cells isolated from chimeric mice nevertheless failed to show the myoblast fusion defect or sarcomere assembly defect in cultured cells [121]. Later work using a conditional knockout of β1 integrins in skeletal muscle did show that integrins are needed for myoblast fusion and sarcomere stability [122]. To explain this discrepancy it has been suggested that compensation mechanisms mask the defect during *in vitro* cell culture but that it is manifested *in vivo*. Likely candidates that probably cooperate in the muscle cells at various stages of myogenesis are the α4, α5 and α7 integrin chains [123]. It is clear that some aspects of integrin function during the complex process of vertebrate myogenesis remain to be clarified.

α5β1 integrin [124] is a major embryonic/foetal muscle integrin during myogenesis *in vivo*, whereas α7β1 is the important adult muscle integrin [125]. In the adult skeletal muscle sarcolemma the α7β1 and dystroglycan receptors interact with different laminin isoforms.

In mouse, the number of laminin isoforms and the number of integrins have thus increased compared to the simpler fruit fly. During mouse myogenesis there are switches in the integrin and laminin isoforms being expressed, reflecting the need to control migration and cell adhesive strength. Except for a greater diversity to allow for different adhesion strengths, a new cell type has invaded the muscle in chordates/vertebrates, the fibroblast. In order to be able to interact with fibrillar collagens the fibroblast integrins have acquired an αI domain in their integrin α-chains. In normal muscle the fibroblasts most likely take part in conditioning the muscle environment, controlling muscle gene expression and appearing as a passive player. In pathological situations the importance of these collagen-binding integrins in controlling the fibrotic response remains to be defined, but it is predicted to be substantial.

In the muscle sarcolemma integrins fulfil their functions at the junctions, whereas dystroglycan forms lateral contacts. Gene therapy approaches aimed at strengthening the muscle-basement membrane contact have been predicted to entail difficulties, mainly due to the delicate balance that exists between the laminin receptors in the lateral sarcolemma and in the junctions. Cell adhesion therapy in muscle is a challenging task that will require careful planning, including consideration of the effects of the various spliced forms of α7 and β1 integrins in this scenario [126].

One aspect of muscle formation in vertebrates of which very little is known is muscle attachment site (MAS) formation at the tendon interface. In muscle, as in many other tissues, fibroblasts have received little attention when it comes to tissue function. It has been recognized in stem cell biology that some stem cells use bi-directional signalling to establish a fibroblast stem cell niche [127] in order to maintain stem cell multipotency, and similar bi-directional signalling between cell types might also extend to muscle–tendon attachment sites. In skeletal muscle the muscle fibres make contact with the tendons at the myotendinous junctions (MTJ), but surprisingly little is known about how these contacts are made at the tendon side. Recent work with the transcription factor scleraxis that is expressed in the tendon/ligament has shed some light on tendon formation, but has also highlighted its complexity [128,129]. Although tendon and muscle can form independently of each other (to different degrees in muscles/tendons of different types), the two cell types are clearly dependent on each other for normal development. By analogy with the situation in *Drosophila*, it is possible that the muscle–tendon contact may depend on bi-directional signalling and may even involve direct tendon cell–muscle cell interactions at some stage. It will be important to define the molecules that are concentrated in the junctional fibroblasts at MTJs (Fig. 4), and it will also be interesting to identify the molecules involved in attaching the tendon to the bone. Future experiments will be certain to address this important issue, and the zebrafish might be a good model for studying tendon–bone attachments.

3.4. Outlook for other animal models in the study of integrin function in muscle

In addition to fruit fly and mouse, a number of additional animal models have the potential to be useful in elucidating integrin function during different aspects of myogenesis. Frogs have been extensively used to study the early steps in embryogenesis, including gastrulation [95–98]. Elegant work has led to the identification of fibronectin and of fibronectin-binding integrins as playing a role in organizing fibronectin during gastrulation [96]. A limited number of studies have been performed on integrins and muscle formation in *Xenopus*. One recent study showed that in *Xenopus* integrin-dependent somite formation depends on the proteins FAK and VASP [99]. If this is an evolutionary conserved function of integrins it is difficult to say at present. In mouse, β1 integrin$^{-/-}$ embryos die prior to somitogenesis [100], and the lack of somites in α5$^{-/-}$ embryos is secondary to mesodermal defects [101]. To our knowledge, no specific integrin mutation affecting somitiogenesis specifically is known in mouse. Further studies of early steps of muscle formation might be informative in *Xenopus* (Table 3).

Another model organism that has seen a big increase in interest during the post-genomic era is the zebrafish (*D. rerio*) [102,103], the embryos of which are transparent, allowing easy visualization, and a number of mutants are available. The one complication that arises in genetic studies is related to ancient genome duplication, causing the

Table 3
Evolutionary conserved functions of integrins in skeletal muscle.

Model organism	Integrin function in skeletal muscle	Evolutionary conserved	Usefulness of model
Drosophila	Cell adhesion (mechanical link) Sarcomere structure Myogenic differentiation	Yes	Studies of molecular mechanisms.
Xenopus	nd	nd	Early muscle development.
Zebrafish	Cell adhesion (somite border integrity)	?	Studies of muscle tendon attachments. Fin and muscle regeneration.
Mouse	Cell adhesion (Mechanical link) Sarcomere structure Myogenic differentiation	Yes	Muscular dystrophy models.

nd; not determined.

existence of multiple copies of some genes, which in many cases then seem to divide the functions between them. This always has to be borne in mind when trying to understand gene function in the zebrafish. Data on integrins in zebrafish are relatively scarce (50 references in Pubmed Oct. 2008), but one integrin that has been studied in some detail is the integrin α5 orthologue (see below) [104,105]. The promising nature of this organism is illustrated in a recent paper in which a mutation screening focused on heart function identified laminin α4 and ILK as candidate disease genes, a situation which was later validated in human patients [106]. Regarding skeletal muscle formation in zebrafish some studies have started to emerge on proteins previously implicated in human muscle disease [107,108]. Although some orthologues of muscular dystrophy genes are duplicated in the zebrafish, recent data on a dystrophin mutant fish (*sapje*) and a laminin α2 mutant fish (*candyfloss*) have offered new insight into the early steps of muscle disease [109]. In the laminin α2 mutation *candyfloss*, muscle fibre detachment with retained sarcolemma integrity was observed, suggesting that laminin α2 is important for extracellular matrix integrity and that the detachment is an early step of the pathological process before the muscle fibres start to disintegrate. No information on zebrafish integrin α7 mutations is available yet and the data on integrins in muscle in zebrafish is thus limited.

Integrin α5β1 mutations affect early muscle development and closer analysis suggest that this integrin is needed to maintain somite borders during the early steps of muscle formation[104]. Members of the TGF-β family (Activin βA) have been shown to be active during fin regeneration [110], and should offer a good system for studying integrin involvement in muscle regeneration. Finally, several mutations exist in the zebrafish mesoderm, which also affect the tendons, and some of these mutants might be interesting for studying the possible role of integrins in muscle–tendon attachments [111–113].

4. Summary

In summary, we have shown that integrins have diversified and subspecialized during evolution. Current sequence data show that the origin of human-type integrin α and β subunits arose after the divergence of the tunicates but orthologues, including multiple isoforms, are present in fish species. Analysis of sequence data from the genomes of shark species, the Florida lancelet and the sea lamprey may in the very near future clarify the likely point in chordate evolution when human-type homologues first arose. Key domains comprising the ectodomain of the α and β subunits have early origins, with examples found in bacteria, whereas the origin and function of intact α and β integrin subunits extend at least to the very earliest animals such as sponges. The I domain as an additional domain in some integrin α subunits is a relatively late addition, found in the urochordates, whereas they are not found in echinoderms. Integrins with the I domain in tunicates are not orthologous to the vertebrate integrins having the I domain; in the later, there has been a clear differentiation into two groups, one set recognizing cells of the immune system (e.g., αLβ2) and another that functions as collagen receptors (e.g. α2β1).

At least one α subunit and one β subunit exist in sponges (e.g. *G. cydonium*), which lack tissues, muscle and nerves, but do have an embryonic state; already in cnidarians a network of nerves appear and both striated and smooth muscles have been observed. Characteristic of the diverging metazoans is this increase in complexity that probably has required additional integrins. As reflected in *Drosophila* with 5 α subunits and 2 β subunits and human with 18 α subunits and 8 β subunits, the integrin sequences, structures and their cellular functions have clearly evolved as metazoan organisms gained increasingly complex nervous systems, musculature, tissues and organs, a circulatory system and development of the adaptive immune system to complement the innate immunity present in invertebrates. Thus, the increasing complexity of the metazoans appears to have gone hand-in-hand with an increase in the number of integrin subunits and the heterodimers that can be formed providing a means for their functional diversification.

Skeletal muscle is one of the oldest features of the metazoans as motility is a key feature of all animals at least during some stage of development. In *Drosophila*, the embryonic muscles are first tested for functionality when the embryos flex the muscles midway through embryogenesis, and integrins are necessary to form contacts between muscle cells and the cuticular exoskeleton. In vertebrates, muscle is integrated with a skeletal system where interstitial collagens appear as major proteinacious building blocks of tendons, cartilage and bone. In order to be able to interact with these components, fibroblasts, chondrocytes and osteoblasts use integrins that, in contrast to *Drosophila* integrins, have picked up an αI domain in order to anchor cells into this collagenous matrix [38,39]. During inflammation or muscle damage, infiltrating leukocytes and macrophages use αI domain-containing β2 integrins to move into the muscle tissue.

The integrin linkage provides an attachment mechanism at muscle endpoints both in fruit flies and vertebrates. Surprisingly, in vertebrates some aspects of this linkage still remain unclear. Whereas the integrin αPS2βPS or α7β1 integrins forms the muscle integrin linkage to laminin-containing basement membranes in adult invertebrate and vertebrate muscles respectively, virtually nothing is known about the linkage from the vertebrate tendon fibroblasts to the muscle attachment points. The future will tell if the vertebrate muscle cell-endoskeleton linkage shows conserved features at the molecular level with the invertebrate muscle cell-exoskeleton linkage and also involves integrins. It will also be interesting to see if the similarities to *Drosophila* muscle junctions extend to the perdido-mediated mechanisms. Zebrafish might in the future be a useful model since several mesoderm mutants exist that might shed light on the molecular mechanisms involved in muscle–tendon linkages. Regarding the usefulness of the discussed animal models in helping to elucidate muscle disease mechanisms, zebrafish might also prove to be useful and in a not too distant future zebrafish might give useful clues to potential molecular targets to be stimulated in therapeutic approaches to muscle regeneration.

We have discussed in this review the molecular principles that have evolved within the integrin protein family and the molecular strategies that have been retained. As in all families, the processes of exploring the past and keeping the family heritage alive will continue to shed light on why the current family members are as they are.

References

[1] R.O. Hynes, Cell adhesion: old and new questions, Trends Cell Biol. 9 (1999) M33–M37.
[2] R.O. Hynes, Q. Zhao, The evolution of cell adhesion, J. Cell. Biol. 150 (2000) F89–F96.
[3] C. Bokel, N.H. Brown, Integrins in development: moving on, responding to, and sticking to the extracellular matrix, Dev. Cell. 3 (2002) 311–321.
[4] J.M. Linton, G.R. Martin, L.F. Reichardt, The ECM protein nephronectin promotes kidney development via integrin alpha8beta1-mediated stimulation of Gdnf expression, Development 134 (2007) 2501–2509.
[5] C.-Q. Zhu, S. Popova, E.R.S. Brown, D. Barsyte-Lovejoy, R. Navab, W. Shih, M. Li, I. Jurisica, L. Penn, D. Gullberg, M.S. Tsao, Integrin alpha11 regulates insulin-like growth factor(IGF)-2 expression in fibroblasts and tumorigenicity of human non-small cell lung cancer cells, Proc. Natl. Acad. Sci. U. S. A 104 (2007) 11754–11759.
[6] L.E. Reynolds, F.J. Conti, M. Lucas, R. Grose, S. Robinson, M. Stone, G. Saunders, C. Dickson, R.O. Hynes, A. Lacy-Hulbert, K. Hodivala-Dilke, Accelerated re-epithelialization in beta3-integrin-deficient- mice is associated with enhanced TGF-beta1 signaling, Nat. Med. 11 (2005) 167–174.
[7] M. Van Doren, Much HUBbub about stem-cell niches, Nat. Cell Biol. 9 (2007) 1344–1345.
[8] G. Tanentzapf, D. Devenport, D. Godt, N.H. Brown, Integrin-dependent anchoring of a stem-cell niche, Nat. Cell Biol. 9 (2007) 1413–1418.
[9] N.H. Brown, Cell–cell adhesion via the ECM: integrin genetics in fly and worm, Matrix Biol. 19 (2000) 191–201.
[10] R.O. Hynes, Integrins: bidirectional, allosteric signaling machines, Cell 110 (2002) 673–687.

[11] J. Heino, R.A. Ignotz, M.E. Hemler, C. Crouse, J. Massague, Regulation of cell adhesion receptors by transforming growth factor-beta. Concomitant regulation of integrins that share a common beta 1 subunit, J. Biol. Chem. 264 (1989) 380–388.

[12] D. Sheppard, D.S. Cohen, A. Wang, M. Busk, Transforming growth factor beta differentially regulates expression of integrin subunits in guinea pig airway epithelial cells, J. Biol. Chem. 267 (1992) 17409–17414.

[13] M.S. Johnson, D. Tuckwell, Evolution of Integrin I Domains, Kluwer Academic/Plenum Publishers, Georgetown, Texas, 2003.

[14] C.A. Whittaker, R.O. Hynes, Distribution and evolution of von Willebrand/integrin A domains: widely dispersed domains with roles in cell adhesion and elsewhere, Mol. Biol. Cell. 13 (2002) 3369–3387.

[15] N.H. Brown, An integrin chicken and egg problem: which comes first, the extracellular matrix or the cytoskeleton? Curr. Opin. Cell. Biol. 12 (2000) 629–633.

[16] C.E.S. Consortium, Genome sequence of the nematode *C. elegans*: a platform for investigating biology, Science 282 (1998) 2012–2018.

[17] S.N. Gettner, C. Kenyon, L.F. Reichardt, Characterization of beta pat-3 heterodimers, a family of essential integrin receptors in *C. elegans*, J. Cell. Biol. 129 (1995) 1127–1141.

[18] M. Labouesse, E. Georges-Labouesse, *C. elegans* integrins, in: Erick H.J. Danen (Ed.), Integrins and Development, Landes Bioscience, 2006, p. 234.

[19] M.C. Brabant, D.L. Brower, PS2 integrin requirements in *Drosophila* embryo and wing morphogenesis, Dev. Biol. 157 (1993) 49–59.

[20] N.H. Brown, Null mutations in the alpha PS2 and beta PS integrin subunit genes have distinct phenotypes, Development 120 (1994) 1221–1231.

[21] M. Leptin, T. Bogaert, R. Lehmann, M. Wilcox, The function of PS integrins during *Drosophila* embryogenesis, Cell 56 (1989) 401–408.

[22] G.H. Yee, R.O. Hynes, A novel, tissue-specific integrin subunit, beta nu, expressed in the midgut of *Drosophila melanogaster*, Development 118 (1993) 845–858.

[23] M.D. Adams, S.E. Celniker, R.A. Holt, C.A. Evans, J.D. Gocayne, P.G. Amanatides, S.E. Scherer, P.W. Li, R.A. Hoskins, R.F. Galle, R.A. George, S.E. Lewis, S. Richards, M. Ashburner, S.N. Henderson, G.G. Sutton, J.R. Wortman, M.D. Yandell, Q. Zhang, L.X. Chen, R.C. Brandon, Y.H. Rogers, R.G. Blazej, M. Champe, B.D. Pfeiffer, K.H. Wan, C. Doyle, E.G. Baxter, G. Helt, C.R. Nelson, G.L. Gabor, J.F. Abril, A. Agbayani, H.J. An, C. Andrews-Pfannkoch, D. Baldwin, R.M. Ballew, A. Basu, J. Baxendale, L. Bayraktaroglu, E.M. Beasley, K.Y. Beeson, P.V. Benos, B.P. Berman, D. Bhandari, S. Bolshakov, D. Borkova, M.R. Botchan, J. Bouck, P. Brokstein, P. Brottier, K.C. Burtis, D.A. Busam, H. Butler, E. Cadieu, A. Center, I. Chandra, J.M. Cherry, S. Cawley, C. Dahlke, L.B. Davenport, P. Davies, B. de Pablos, A. Delcher, Z. Deng, A.D. Mays, I. Dew, S.M. Dietz, K. Dodson, L.E. Doup, M. Downes, S. Dugan-Rocha, B.C. Dunkov, P. Dunn, K.J. Durbin, C.C. Evangelista, C. Ferraz, S. Ferriera, W. Fleischmann, C. Fosler, A.E. Gabrielian, N.S. Garg, W.M. Gelbart, K. Glasser, A. Glodek, F. Gong, J.H. Gorrell, Z. Gu, P. Guan, M. Harris, N.L. Harris, D. Harvey, T.J. Heiman, J.R. Hernandez, J. Houck, D. Hostin, K.A. Houston, T.J. Howland, M.H. Wei, C. Ibegwam, M. Jalali, F. Kalush, G.H. Karpen, Z. Ke, J.A. Kennison, K.A. Ketchum, B.E. Kimmel, C.D. Kodira, C. Kraft, S. Kravitz, D. Kulp, Z. Lai, P. Lasko, Y. Lei, A.A. Levitsky, J. Li, Z. Li, Y. Liang, X. Lin, X. Liu, B. Mattei, T.C. McIntosh, M.P. McLeod, D. McPherson, G. Merkulov, N.V. Milshina, C. Mobarry, J. Morris, A. Moshrefi, S.M. Mount, M. Moy, B. Murphy, L. Murphy, D.M. Muzny, D.L. Nelson, D.R. Nelson, K.A. Nelson, K. Nixon, D.R. Nusskern, J.M. Pacleb, M. Palazzolo, G.S. Pittman, S. Pan, J. Pollard, V. Puri, M.G. Reese, K. Reinert, K. Remington, R.D. Saunders, F. Scheeler, H. Shen, B.C. Shue, I. Siden-Kiamos, M. Simpson, M.P. Skupski, T. Smith, E. Spier, A.C. Spradling, M. Stapleton, R. Strong, E. Sun, R. Svirskas, C. Tector, R. Turner, E. Venter, A.H. Wang, X. Wang, Z.Y. Wang, D.A. Wassarman, G.M. Weinstock, J. Weissenbach, S.M. Williams, T. Woodage, K.C. Worley, D. Wu, S. Yang, Q.A. Yao, J. Ye, R.F. Yeh, J.S. Zaveri, M. Zhan, G. Zhang, Q. Zhao, L. Zheng, X.H. Zheng, F.N. Zhong, W. Zhong, X. Zhou, S. Zhu, X. Zhu, H.O. Smith, R.A. Gibbs, E.W. Myers, G.M. Rubin, J.C. Venter, The genome sequence of *Drosophila melanogaster*, Science 287 (2000) 2185–2195.

[24] A. Prokop, M.D. Martin-Bermudo, M. Bate, N.H. Brown, Absence of PS integrins or laminin A affects extracellular adhesion, but not intracellular assembly, of hemiadherens and neuromuscular junctions in *Drosophila* embryos, Dev. Biol. 196 (1998) 58–76.

[25] K.A. Stark, G.H. Yee, C.E. Roote, E.L. Williams, S. Zusman, R.O. Hynes, A novel alpha integrin subunit associates with betaPS and functions in tissue morphogenesis and movement during *Drosophila* development, Development 124 (1997) 4583–4594.

[26] R. Ewan, J. Huxley-Jones, A.P. Mould, M.J. Humphries, D.L. Robertson, R.P. Boot-Handford, The integrins of the urochordate *Ciona intestinalis* provide novel insights into the molecular evolution of the vertebrate integrin family, BMC Evol. Biol. 5 (2005) 31.

[27] A.L. Hughes, Evolution of the integrin alpha and beta protein families, J. Mol. Evol. 52 (2001) 63–72.

[28] M. Huhtala, J. Heino, D. Casciari, A. de Luise, M.S. Johnson, Integrin evolution: insights from ascidian and teleost fish genomes, Matrix Biol. 24 (2005) 83–95.

[29] Y. Sasakura, E. Shoguchi, N. Takatori, I.A. Wada, Y. Meinertzhagen, N. Satou, A. Satoh, A genomewide survey of developmentally relevant genes in *Ciona intestinalis*. X. Genes for cell junctions and extracellular matrix, Dev. Genes Evol. 213 (2003) 303–313.

[30] P. Gotwals, L.I. Fessler, M. Wehrli, R. Hynes, *Drosophila* PS1 integrin is a laminin receptor and differs in ligand specificity from PS2, Proc. Natl. Acad. Sci. U. S. A. 91 (1994) 11447–11451.

[31] Z. Pancer, M. Kruse, I. Muller, W.E. Muller, On the origin of Metazoan adhesion receptors: cloning of integrin alpha subunit from the sponge *Geodia cydonium*, Mol. Biol. Evol. 14 (1997) 391–398.

[32] F.J. Fogerty, L.I. Fessler, T.A. Bunch, Y. Yaron, C.G. Parker, R.E. Nelson, D.L. Brower, D. Gullberg, J.H. Fessler, Tiggrin, a novel *Drosophila* extracellular matrix protein that functions as a ligand for *Drosophila* αPS2βPS3 integrins. Development 120 (1994) 1747–1758.

[33] J.T. Yang, H. Rayburn, R.O. Hynes, Embryonic mesodermal defects in a5 integrin-deficient mice, Development 119 (1993) 1093–1105.

[34] S. Pahler, B. Blumbach, I. Muller, W.E. Muller, Putative multiadhesive protein from the marine sponge *Geodia cydonium*: cloning of the cDNA encoding a fibronectin-, an SRCR-, and a complement control protein module, J. Exp. Zool. 282 (1998) 332–343.

[35] T.A. Springer, J. Zhu, T. Xiao, Structural basis for distinctive recognition of fibrinogen gammaC peptide by the platelet integrin alphaIIbbeta3, J. Cell. Biol. 182 (2008) 791–800.

[36] J.S. Munger, X. Huang, H. Kawakatsu, M.J. Griffiths, S.L. Dalton, J. Wu, J.F. Pittet, N. Kaminski, C. Garat, M.A. Matthay, D.B. Rifkin, D. Sheppard, The integrin avb6 binds and activates latent TGF β1: a mechanism for regulating pulmonary inflammation and fibrosis, Cell 96 (1999) 319–328.

[37] N.E. Vlahakis, B.A. Young, A. Atakilit, A.E. Hawkridge, R.B. Issaka, N. Boudreau, D. Sheppard, Integrin alpha9beta1 directly binds to vascular endothelial growth factor (VEGF)-A and contributes to VEGF-A-induced angiogenesis, J. Biol. Chem. 282 (2007) 15187–15196.

[38] J. Heino, The collagen family members as cell adhesion proteins, Bioessays 29 (2007) 1001–1010.

[39] S.N. Popova, E. Lundgren-Akerlund, H. Wiig, D. Gullberg, Physiology and pathology of collagen receptors, Acta. Physiol. (Oxf). 190 (2007) 179–187.

[40] D.M. Rose, R. Alon, M.H. Ginsberg, Integrin modulation and signaling in leukocyte adhesion and migration, Immunol. Rev. 218 (2007) 126–134.

[41] K. Suzuki, T. Okuno, M. Yamamoto, R.J. Pasterkamp, N. Takegahara, H. Takamatsu, T. Kitao, J. Takagi, P.D. Rennert, A.L. Kolodkin, A. Kumanogoh, H. Kikutani, Semaphorin 7A initiates T-cell-mediated inflammatory responses through alpha1beta1 integrin, Nature 446 (2007) 680–684.

[42] M. Tulla, M. Huhtala, J. Jaalinoja, J. Kapyla, R.W. Farndale, L. Ala-Kokko, M.S. Johnson, J. Heino, Analysis of an ascidian integrin provides new insight into early evolution of collagen recognition, FEBS Lett. 581 (2007) 2434–2440.

[43] J. Emsley, C.G. Knight, R.W. Farndale, M.J. Barnes, R.C. Liddington, Structural basis of collagen recognition by integrin α2β1, Cell 101 (2000) 47–56.

[44] W.M. Zhang, J. Kapyla, J.S. Puranen, C.G. Knight, C.F. Tiger, O.T. Pentikainen, M.S. Johnson, R.W. Farndale, J. Heino, D. Gullberg, α11β1 integrin recognizes the GFOGER sequence in interstitial collagens, J. Biol. Chem. 278 (2003) 7270–7277.

[45] S. Miyazawa, K. Azumi, M. Nonaka, Cloning and characterization of integrin alpha subunits from the solitary ascidian, *Halocynthia roretzi*, J. Immunol. 166 (2001) 1710–1715.

[46] S. Miyazawa, M. Nonaka, Characterization of novel ascidian beta integrins as primitive complement receptor subunits, Immunogenetics 55 (2004) 836–844.

[47] M.G. Klotz, D.J. Arp, P.S. Chain, A.F. El-Sheikh, L.J. Hauser, N.G. Hommes, F.W. Larimer, S.A. Malfatti, J.M. Norton, A.T. Poret-Peterson, L.M. Vergez, B.B. Ward, Complete genome sequence of the marine, chemolithoautotrophic, ammonia-oxidizing bacterium *Nitrosococcus oceani* ATCC 19707, Appl. Environ. Microbiol. 72 (2006) 6299–6315.

[48] W. Wimmer, B. Blumbach, B. Diehl-Seifert, C. Koziol, R. Batel, R. Steffen, I.M. Muller, W.E. Muller, Increased expression of integrin and receptor tyrosine kinase genes during autograft fusion in the sponge *Geodia cydonium*, Cell. Adhes. Commun. 7 (1999) 111–124.

[49] 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 aVb3, Science 294 (2001) 339–345.

[50] 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.

[51] M.A. Arnaout, B. Mahalingam, J.P. Xiong, Integrin structure, allostery, and bidirectional signaling, Annu. Rev. Cell. Dev. Biol. 21 (2005) 381–410.

[52] J.P. Xiong, T. Stehle, S.L. Goodman, M.A. Arnaout, New insights into the structural basis of integrin activation, Blood 102 (2003) 1155–1159.

[53] 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 alpha Vbeta3 in complex with an Arg-Gly-Asp ligand, Science 296 (2002) 151–155.

[54] B.D. Adair, J.P. Xiong, C. Maddock, S.L. Goodman, M.A. Arnaout, M. Yeager, Three-dimensional EM structure of the ectodomain of integrin {alpha}V{beta}3 in a complex with fibronectin, J. Cell. Biol. 168 (2005) 1109–1118.

[55] L. Xing, M. Huhtala, V. Pietiainen, J. Kapyla, K. Vuorinen, V. Marjomaki, J. Heino, M.S. Johnson, T. Hyypia, R.H. Cheng, Structural and functional analysis of integrin alpha2I domain interaction with echovirus 1, J. Biol. Chem. 279 (2004) 11632–11638.

[56] N. Nishida, C. Xie, M. Shimaoka, Y. Cheng, T. Walz, T.A. Springer, Activation of leukocyte beta2 integrins by conversion from bent to extended conformations, Immunity 25 (2006) 583–594.

[57] D. Tuckwell, Evolution of von Willebrand factor A (VWA) domains, Biochem. Soc. Trans. 27 (1999) 835–840.

[58] A.P. Mould, J.A. McLeish, J. Huxley-Jones, A.C. Goonesinghe, A.F. Hurlstone, R.P. Boot-Handford, M.J. Humphries, Identification of multiple integrin beta1 homologs in zebrafish (*Danio rerio*), BMC. Cell. Biol. 7 (2006) 24.

[59] P.C. Donoghue, M.A. Purnell, Genome duplication, extinction and vertebrate evolution, Trends. Ecol. Evol. 20 (2005) 312–319.

[60] W.C. Parks, What is the alpha2beta1 integrin doing in the epidermis? J. Invest. Dermatol. 127 (2007) 264–266.

[61] A. Aouacheria, C. Cluzel, C. Lethias, M. Gouy, R. Garrone, J.Y. Exposito, Invertebrate data predict an early emergence of vertebrate fibrillar collagen clades and an anti-incest model, J. Biol. Chem. 279 (2004) 47711–47719.
[62] R.P. Boot-Handford, D.S. Tuckwell, Fibrillar collagen: the key to vertebrate evolution? A tale of molecular incest, Bioessays 25 (2003) 142–151.
[63] R. Czaker, Extracellular matrix (ECM) components in a very primitive multicellular animal, the dicyemid mesozoan *Kantharella antarctica*, Anat. Rec. 259 (2000) 52–59.
[64] R. Har-el, M.L. Tanzer, Extracellular matrix. 3: Evolution of the extracellular matrix in invertebrates, Faseb J. 7 (1993) 1115–1123.
[65] J. Bienkowska, M. Cruz, A. Atiemo, R. Handin, R. Liddington, The von Willebrand factor A3 domain does not contain a metal ion-dependent adhesion site motif, J. Biol. Chem. 272 (1997) 25162–25167.
[66] R. Celikel, K.I. Varughese, Madhusudan, A. Yoshioka, J. Ware, Z.M. Ruggeri, Crystal structure of the von Willebrand factor A1 domain in complex with the function blocking NMC-4 Fab, Nat. Struct. Biol. 5 (1998) 189–194.
[67] E.G. Huizinga, R. Martijn van der Plas, J. Kroon, J.J. Sixma, P. Gros, Crystal structure of the A3 domain of human von Willebrand factor: implications for collagen binding, Structure 5 (1997) 1147–1156.
[68] A. Qu, D.J. Leahy, Crystal structure of the I-domain from the CD11a/CD18 (LFA-1, alpha L beta 2) integrin, Proc. Natl. Acad. Sci. U. S. A. 92 (1995) 10277–10281.
[69] J.O. Lee, P. Rieu, M.A. Arnaout, R. Liddington, Crystal structure of the A domain from the alpha subunit of integrin CR3 (CD11b/CD18), Cell 80 (1995) 631–638.
[70] T.A. Salminen, Y. Nymalm, J. Kankare, J. Kapyla, J. Heino, M.S. Johnson, Production, crystallization and preliminary X-ray analysis of the human integrin alpha1 I domain, Acta. Crystallogr. D. Biol. Crystallogr. 55 (1999) 1365–1367.
[71] J. Emsley, S.L. King, J.M. Bergelson, R.C. Liddington, Crystal structure of the I domain from integrin $\alpha 2\beta 1$, J. Biol. Chem. 272 (1997) 28512–28517.
[72] M.R. Custodio, G. Imsiecke, R. Borojevic, B. Rinkevich, A. Rogerson, W.E. Muller, Evolution of cell adhesion systems: evidence for Arg-Gly-Asp-mediated adhesion in the protozoan *Neoparamoeba aestuarina*, J. Eukaryot. Microbiol. 42 (1995) 721–724.
[73] N.H. Brown, Integrins hold *Drosophila* together, Bioessays 15 (1993) 383–390.
[74] S. Murakami, D. Umetsu, Y. Maeyama, M. Sato, S. Yoshida, T. Tabata, Focal adhesion kinase controls morphogenesis of the *Drosophila* optic stalk, Development 134 (2007) 1539–1548.
[75] A. Stevens, J.R. Jacobs, Integrins regulate responsiveness to slit repellent signals, J. Neurosci. 22 (2002) 4448–4455.
[76] D.L. Brower, Platelets with wings: the maturation of *Drosophila* integrin biology, Curr. Opin. Cell. Biol. 15 (2003) 607–613.
[77] M. Ruiz-Gomez, Muscle patterning and specification in *Drosophila*, Int. J. Dev. Biol. 42 (1998) 283–290.
[78] B.M. Paterson, M. Shirakata, S. Nakamura, C. Dechesne, U. Walldorf, J. Eldridge, A. Dubendorfer, M. Frasch, W.J. Gehring, Isolation and functional comparison of Dmyd, the *Drosophila* homologue of the vertebrate myogenic determination genes, with CMD1, Symp. Soc. Exp. Biol. 46 (1992) 89–109.
[79] M. Bate, The embryonic development of larval muscles in *Drosophila*, Development 110 (1990) 791–804.
[80] F. Schnorrer, B.J. Dickson, Muscle building; mechanisms of myotube guidance and attachment site selection, Dev. Cell. 7 (2004) 9–20.
[81] S. Zusman, R.S. Patel-King, C. Ffrench-Constant, R.O. Hynes, Requirements for integrins during *Drosophila* development, Development 108 (1990) 391–402.
[82] A.J. MacKrell, B. Blumberg, S.R. Haynes, J.H. Fessler, The lethal myospheroid gene of *Drosophila* encodes a membrane protein homologous to vertebrate integrin beta subunits, Proc. Natl. Acad. Sci. U. S. A. 85 (1988) 2633–2637.
[83] T.F. Wright, The phenogenetics of the embryonic mutant, lethal myospheroid, in *Drosophila melanogaster*, J. Exp. Zool. 143 (1960) 77–99.
[84] T. Volk, L.I. Fessler, J.H. Fessler, A role for integrin in the formation of sarcomeric cytoarchitecture. Cell 63 (1991) 525–536.
[85] T. Bogaert, N. Brown, M. Wilcox, The *Drosophila* PS2 antigen is an invertebrate integrin that, like the fibronectin receptor, becomes localized to muscle attachments, Cell 51 (1987) 929–940.
[86] M. Kusche-Gullberg, K. Garrison, A.J. MacKrell, L.I. Fessler, J.H. Fessler, Laminin A chain: expression during *Drosophila* development and genomic sequence, EMBO J. 11 (1992) 4519–4527.
[87] S. Baumgartner, Identification of a *Drosophila* homologue of the laminin a2 chain, FECTS meeting report, Munich (1996).
[88] D. Martin, S. Zusman, X. Li, E.L. Williams, N. Khare, S. DaRocha, R. Chiquet-Ehrismann, S. Baumgartner, wing blister, a new *Drosophila* laminin alpha chain required for cell adhesion and migration during embryonic and imaginal development, J. Cell. Biol. 145 (1999) 191–201.
[89] T. Yarnitzky, T. Volk, Laminin is required for heart, somatic muscles, and gut development in the *Drosophila* embryo, Dev. Biol. 169 (1995) 609–618.
[90] B. Chanana, R. Graf, T. Koledachkina, R. Pflanz, G. Vorbruggen, alpha(PS2) integrin-mediated muscle attachment in *Drosophila* requires the ECM protein Thrombospondin, Mech. Dev. 124 (2007) 463–475.
[91] A. Subramanian, B. Wayburn, T. Bunch, T. Volk, Thrombospondin-mediated adhesion is essential for the formation of the myotendinous junction in *Drosophila*, Development 134 (2007) 1269–1278.
[92] B. Estrada, S.S. Gisselbrecht, A.M. Michelson, The transmembrane protein Perdido interacts with Grip and integrins to mediate myotube projection and attachment in the *Drosophila* embryo, Development 134 (2007) 4469–4478.
[93] A. Nystrom, J. Holmblad, F. Pedrosa-Domellof, T. Sasaki, M. Durbeej, Extraocular muscle is spared upon complete laminin alpha2 chain deficiency: comparative expression of laminin and integrin isoforms, Matrix Biol. 25 (2006) 382–385.
[94] D. Gullberg, L.I. Fessler, J.H. Fessler, Differentiation, extracellular matrix synthesis, and integrin assembly by *Drosophila* embryo cells cultured on vitronectin and laminin substrates, Dev. Dynamics 199 (1994) 116–128.
[95] D. Alfandari, H. Cousin, A. Gaultier, B.G. Hoffstrom, D.W. DeSimone, Integrin alpha5beta1 supports the migration of *Xenopus* cranial neural crest on fibronectin, Dev. Biol. 260 (2003) 449–464.
[96] L.A. Davidson, B.G. Hoffstrom, R. Keller, D.W. DeSimone, Mesendoderm extension and mantle closure in *Xenopus laevis* gastrulation: combined roles for integrin alpha(5)beta(1), fibronectin, and tissue geometry, Dev. Biol. 242 (2002) 109–129.
[97] J.W. Ramos, C.A. Whittaker, D.W. DeSimone, Integrin-dependent adhesive activity is spatially controlled by inductive signals at gastrulation, Development 122 (1996) 2873–2883.
[98] J.C. Smith, K. Symes, R.O. Hynes, D. DeSimone, Mesoderm induction and the control of gastrulation in *Xenopus laevis*: the roles of fibronectin and integrins, Development 108 (1990) 229–238.
[99] K.A. Kragtorp, J.R. Miller, Regulation of somitogenesis by Ena/VASP proteins and FAK during *Xenopus* development, Development 133 (2006) 685–695.
[100] R. Fässler, M. Meyer, Consequences of lack of b1 integrin gene expression in mice, Genes Dev. 9 (1995) 1896–1908.
[101] J.T. Yang, B.L. Bader, J.A. Kreidberg, M. Ullman-Cullere, J.E. Trevithick, R.O. Hynes, Overlapping and independent functions of fibronectin receptor integrins in early mesodermal development, Dev. Biol. 215 (1999) 264–277.
[102] C.H. Hsu, Z.H. Wen, C.S. Lin, C. Chakraborty, The zebrafish model: use in studying cellular mechanisms for a spectrum of clinical disease entities, Curr. Neurovasc. Res. 4 (2007) 111–120.
[103] D. Beis, D.Y. Stainier, In vivo cell biology: following the zebrafish trend, Trends Cell Biol. 16 (2006) 105–112.
[104] S. Koshida, Y. Kishimoto, H. Ustumi, T. Shimizu, M. Furutani-Seiki, H. Kondoh, S. Takada, Integrinalpha5-dependent fibronectin accumulation for maintenance of somite boundaries in zebrafish embryos, Dev. Cell. 8 (2005) 587–598.
[105] J.G. Crump, M.E. Swartz, C.B. Kimmel, An integrin-dependent role of pouch endoderm in hyoid cartilage development, PLoS Biol. 2 (2004) E244.
[106] R. Knoll, R. Postel, J. Wang, R. Kratzner, G. Hennecke, A.M. Vacaru, P. Vakeel, C. Schubert, K. Murthy, B.K. Rana, D. Kube, G. Knoll, K. Schafer, T. Hayashi, T. Holm, A. Kimura, N. Schork, M.R. Toliat, P. Nurnberg, H.P. Schultheiss, W. Schaper, J. Schaper, E. Bos, J. Den Hertog, F.J. van Eeden, P.J. Peters, G. Hasenfuss, K.R. Chien, J. Bakkers, Laminin-alpha4 and integrin-linked kinase mutations cause human cardiomyopathy via simultaneous defects in cardiomyocytes and endothelial cells, Circulation 116 (2007) 515–525.
[107] L.S. Steffen, J.R. Guyon, E.D. Vogel, R. Beltre, T.J. Pusack, Y. Zhou, L.I. Zon, L.M. Kunkel, Zebrafish orthologs of human muscular dystrophy genes, BMC Genomics 8 (2007) 79.
[108] J.R. Guyon, L.S. Steffen, M.H. Howell, T.J. Pusack, C. Lawrence, L.M. Kunkel, Modeling human muscle disease in zebrafish, Biochim. Biophys. Acta. 1772 (2007) 205–215.
[109] T.E. Hall, R.J. Bryson-Richardson, S. Berger, A.S. Jacoby, N.J. Cole, G.E. Hollway, J. Berger, P.D. Currie, The zebrafish candyfloss mutant implicates extracellular matrix adhesion failure in laminin alpha2-deficient congenital muscular dystrophy, Proc. Natl. Acad. Sci. U. S. A. 104 (2007) 7092–7097.
[110] A. Jazwinska, R. Badakov, M.T. Keating, Activin-betaA signaling is required for zebrafish fin regeneration, Curr. Biol. 17 (2007) 1390–1395.
[111] C. Thisse, B. Thisse, T.F. Schilling, J.H. Postlethwait, Structure of the zebrafish snail1 gene and its expression in wild-type, spadetail and no tail mutant embryos, Development 119 (1993) 1203–1215.
[112] C. Thisse, B. Thisse, J.H. Postlethwait, Expression of snail2, a second member of the zebrafish snail family, in cephalic mesendoderm and presumptive neural crest of wild-type and spadetail mutant embryos, Dev. Biol. 172 (1995) 86–99.
[113] K.J. Griffin, S.L. Amacher, C.B. Kimmel, D. Kimelman, Molecular identification of spadetail: regulation of zebrafish trunk and tail mesoderm formation by T-box genes, Development 125 (1998) 3379–3388.
[114] T. Bengtsson, A. Aszodi, C. Nicolae, E.B. Hunziker, E. Lundgren-Akerlund, R. Fassler, Loss of $\alpha 10\beta 1$ integrin expression leads to moderate dysfunction of growth plate chondrocytes, J. Cell. Sci. 118 (2005) 929–936.
[115] S.N. Popova, M. Barrzcyk, C. Tiger, W. Beertsen, P. Zigrino, A. Aszodi, N. Miosge, E. Forsberg, D. Gullberg, $\alpha 11\beta 1$ integrin-dependent regulation of periodontal ligament function in the erupting mouse incisor, Mol. Cell. Biol. 27 (2007) 4306–4316.
[116] Y. Takada, X. Ye, S. Simon, The integrins, Genome. Biol. 8 (2007) 215.
[117] C. Chen, D. Sheppard, Identification and molecular characterization of multiple phenotypes in integrin knockout mice, Methods Enzymol. 426 (2007) 291–305.
[118] D.J. Wells, K.E. Wells, What do animal models have to tell us regarding Duchenne muscular dystrophy? Acta. Myol. 24 (2005) 172–180.
[119] G. Chen, I.D. Dimitriou, J. La Rose, S. Ilangumaran, W.C. Yeh, G. Doody, M. Turner, J. Gommerman, R. Rottapel, The 3BP2 adapter protein is required for optimal B-cell activation and thymus-independent type 2 humoral response, Mol. Cell. Biol. 27 (2007) 3109–3122.
[120] Y. Ueki, C.Y. Lin, M. Senoo, T. Ebihara, N. Agata, M. Onji, Y. Saheki, T. Kawai, P.M. Mukherjee, E. Reichenberger, B.R. Olsen, Increased myeloid cell responses to M-CSF and RANKL cause bone loss and inflammation in SH3BP2 "cherubism" mice, Cell 128 (2007) 71–83.
[121] E. Hirsch, L. Lohikangas, D. Gullberg, S. Johansson, R. Fassler, Mouse myoblasts can fuse and form a normal sarcomere in the absence of beta1 integrin expression, J. Cell. Sci. 111 (1998) 2397–2409.
[122] M. Schwander, M. Leu, M. Stumm, O.M. Dorchies, U.T. Ruegg, J. Schittny, U. Muller, $\beta 1$ integrins regulate myoblast fusion and sarcomere assembly, Dev. Cell. 4 (2003) 673–685.

[123] D. Gullberg, Cell biology: the molecules that make muscle, Nature 424 (2003) 138–140.
[124] D. Taverna, M.H. Disatnik, H. Rayburn, R.T. Bronson, J. Yang, T.A. Rando, R.O. Hynes, Dystrophic muscle in mice chimeric for expression of α5 integrin, J. Cell. Biol. 143 (1998) 849–859.
[125] U. Mayer, G. Saher, R. Fassler, A. Bornemann, F. Echtermeyer, H. von der Mark, N. Miosge, E. Poschl, K. von der Mark, Absence of integrin alpha 7 causes a novel form of muscular dystrophy, Nat. Genet. 17 (1997) 318–323.
[126] U. Mayer, Integrins: redundant or important players in skeletal muscle? J. Biol. Chem. 278 (2003) 14587–14590.
[127] S.C. Bendall, M.H. Stewart, P. Menendez, D. George, K. Vijayaragavan, T. Werbowetski-Ogilvie, V. Ramos-Mejia, A. Rouleau, J. Yang, M. Bosse, G. Lajoie, M. Bhatia, IGF and FGF cooperatively establish the regulatory stem cell niche of pluripotent human cells in vitro, Nature 448 (2007) 1015–1021.
[128] N.D. Murchison, B.A. Price, D.A. Conner, D.R. Keene, E.N. Olson, C.J. Tabin, R. Schweitzer, Regulation of tendon differentiation by scleraxis distinguishes force-transmitting tendons from muscle-anchoring tendons, Development 134 (2007) 2697–2708.
[129] B.A. Pryce, A.E. Brent, N.D. Murchison, C.J. Tabin, R. Schweitzer, Generation of transgenic tendon reporters, ScxGFP and ScxAP, using regulatory elements of the scleraxis gene, Dev. Dyn. 236 (2007) 1677–1682.