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Review

The extracellular matrix dimension of skeletal muscle development

Sólveig Thorsteinsdóttir [a,d,*], Marianne Deries [a], Ana Sofia Cachaço [b,d], Fernanda Bajanca [c]

[a] Centro de Biologia Ambiental/Departamento de Biologia Animal, Faculdade de Ciências, Universidade de Lisboa, Lisbon, Portugal
[b] Laboratório de Angiogénese, Centro de Investigação em Patobiologia Molecular (CIPM), Instituto Português de Oncologia de Francisco Gentil and CEDOC, Faculdade de Ciências Médicas, Universidade Nova de Lisboa, Lisbon, Portugal
[c] King's College London, Randall Division for Cell and Molecular Biophysics, London, UK
[d] Instituto Gulbenkian de Ciência, Oeiras, Portugal

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ABSTRACT

Cells anchor to substrates by binding to extracellular matrix (ECM). In addition to this anchoring function however, cell–ECM binding is a mechanism for cells to sense their surroundings and to communicate and coordinate behaviour amongst themselves. Several ECM molecules and their receptors play essential roles in muscle development and maintenance. Defects in these proteins are responsible for some of the most severe muscle dystrophies at every stage of life from neonates to adults. However, recent studies have also revealed a role of cell–ECM interactions at much earlier stages of development as skeletal muscle forms. Here we review which ECM molecules are present during the early phases of myogenesis, how myogenic cells interact with the ECM that surrounds them and the potential consequences of those interactions. We conclude that cell–ECM interactions play significant roles during all stages of skeletal muscle development in the embryo and suggest that this "extracellular matrix dimension" should be added to our conceptual network of factors contributing to skeletal myogenesis.
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Introduction

Skeletal muscle development is a highly regulated process that depends on the interplay between a panoply of cell-autonomous and extrinsic factors. Mesodermal cells commit to the myogenic lineage at certain times and places in the embryo, then differentiate and fuse to form multinucleated myofibres with contractile activity (Biressi et al., 2007; Buckingham, 2001). How undifferentiated cells become muscle cells at the correct time and in the appropriate places in the embryo has been addressed for more than a century (Bardeen, 1900; Williams, 1910). In the last two decades, gene targeting in mice, bead technology for local delivery of growth factors and recombinant gene transfer in chick embryos and mutagenesis or morpholino technology in zebrafish have lead to breakthroughs in our understanding of skeletal muscle development at several levels (Biressi et al., 2007; Bryson-Richardson and Currie, 2008; Buckingham, 2006; Sambasivan and Tajbakhsh, 2007; Scaal and Christ, 2004). These include how the myogenic regulatory factors (MRFs), transcription factors of the helix-loop-helix family and master regulators of skeletal myogenesis, control skeletal muscle commitment and differentiation and how growth factor signalling acts on these MRFs, promoting or inhibiting skeletal myogenesis (Biressi et al., 2007; Buckingham, 2006). The embryonic origin of myogenic precursors has recently been identified and efforts are now ongoing into studying how these precursors give rise to the different types of myoblasts (Biressi et al., 2007; Buckingham and Relaix, 2007). It is gradually being appreciated that skeletal muscle development *in vivo* is much more complex and tightly regulated than myoblast differentiation and fusion *in vitro* (Gullberg et al., 1998; McLennan and Koishi, 2002). In addition, even though we have accumulated considerable knowledge on the process of *in vivo* skeletal myogenesis itself and the role played by transcription factors and growth factor-induced signalling pathways, we know comparatively much less about the role of the extracellular matrix (ECM) in the events that lead mesodermal cells to enter the myogenic lineage and progressively develop into functional muscle fibres.

Our objective in this review is to revisit what we know about the ECM during embryonic myogenesis in the vertebrate embryo by presenting the available data within the current framework of skeletal muscle development *in vivo*. We will cover the early stages of myogenesis using the mouse embryo as a model, but also often referring to the avian systems and occasionally also to zebrafish, whenever appropriate. We will address issues of induction and maintenance of myogenic precursors, their commitment, translocation and differentiation as well as the formation of multinucleated myotubes.

* Corresponding author at: Departamento de Biologia Animal, Faculdade de Ciências, Universidade de Lisboa 1749-016, Lisboa, Portugal. Fax: +351 217500028.
E-mail addresses: solveig@fc.ul.pt (S. Thorsteinsdóttir), mederies@fc.ul.pt (M. Deries), ascachaco@fc.ul.pt (A.S. Cachaço), fernanda.bajanca@kcl.ac.uk (F. Bajanca).

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The extracellular matrix dimension

The extracellular matrix and its relationship with cells in the embryo

The most basic role of the ECM (and the only one recognised for decades) is to provide a supportive scaffold for cells and tissues, promoting cell aggregation and providing a substrate for cell migration (Frantz et al., 2010). ECM assembly and deposition and cell–ECM interactions are the basic motors for polarising cells, separating cell types and supporting short- and long-range cell migrations, consequently shaping the tissues within the developing embryo (Goody and Henry, 2010; Ingber, 2006; Larsen et al., 2006; Rozario and DeSimone, 2010). The ECM is, however, far from being a static entity. It is modified, degraded and reassembled during development and disease as well as during homeostasis (Daley et al., 2008; Frantz et al., 2010; Ghajar and Bissell, 2008). The ECM directly influences cell behaviour through ECM-specific receptors on the cell surface. By binding to ECMs through these receptors, cells sense their surroundings and actively modulate their behaviour depending on its composition (Daley et al., 2008; Ghajar and Bissell, 2008; Ingber, 2006).

Apart from its direct effects, the ECM can also influence cell behaviour indirectly. The ECM can sequester and store soluble growth factors and present them to growth factor receptors on the cell surface at developmentally and/or physiologically relevant times (Kirkpatrick and Selleck, 2007; Rozario and DeSimone, 2010; Selleck, 2000). In this way the ECM may contribute to the establishment of gradients of secreted signalling molecules or can concentrate these factors around certain cell types and facilitate their binding to their receptors (Kirkpatrick and Selleck, 2007; Larsen et al., 2006; Ruoslahti et al., 1992; Selleck, 2000; Schambony et al., 2004; Streuli, 1999). Cell-controlled degradation of the ECM can also release fragments of ECM bound to growth factors and facilitate their binding to signalling receptors on the cell surface (Whitelock et al., 2008).

Thus the ECM and cell–ECM engagement through cell surface receptors not only provides mechanical support, but also signals to the interior of the cell affecting gene expression and such diverse cellular responses as proliferation, polarisation, migration, differentiation, survival and apoptosis, either directly through ECM receptors, or through crosstalk with growth factor signalling pathways (Frantz et al., 2010; Ingber, 2006; Larsen et al., 2006; Rozario and DeSimone, 2010).

Components of the extracellular matrix

The ECM is composed of a variety of glycoproteins and polysaccharide molecules assembled into a macromolecular network (Fig. 1). Both the protein-rich (e.g. collagens, fibronectin, laminins, and tenascins) and polysaccharide-rich (glycosaminoglycans and proteoglycans) molecules of the ECM are secreted by cells and are assembled into an organised meshwork, adapted to the functional requirements of the particular tissue (Frantz et al., 2010). There are two major types of ECMs, the interstitial and pericellular matrices. The first type is the matrix of connective tissue (Fig. 1) and consists of a tissue- and age-specific mixture of a variety of collagen types, elastins, fibronectin, tenascins amongst others, as well as proteoglycans and glycosaminoglycans (Frantz et al., 2010). Pericellular matrices are matrices in close contact with cells and that have a different molecular composition than the surrounding interstitial matrix. The prototype of a pericellular matrix is the basement membrane of epithelial, endothelial, muscle, nerve, and fat cells (Fig. 1). Basement membranes are sheet-like structures, primarily composed of laminins, collagen type IV, nidogen (entactin), and perlecan (a heparan sulphate proteoglycan), and they separate these cell types from the surrounding connective tissue (LeBleu et al., 2007). Laminins are the best studied of these basement membrane components. They are composed of a combination of three chains (α, β and γ; Fig. 1) which exist in different forms, forming at least 16 different laminins (Table 1). Basement membranes are present in the embryo from the earliest stages of development (Colognato and Yurchenco, 2000; Ekblom et al., 2003; Yurchenco et al., 2004) and are essential for embryo viability past E5.5 (Smyth et al., 1999). Another important type of pericellular matrix is the fibronectin matrix of embryonic tissues. Fibronectin matrices are formed by an active cell-based process, where the globular fibronectin molecule is unfolded by specific integrin receptors exposing fibronectin–fibronectin binding sites within the molecule and leading to crosslinking and the assembly of a fibrillar matrix (Mao and Schwarzbauer, 2005; Singh et al., 2010). Fibronectin has traditionally been considered as a component of the mesenchymal ECM, promoting mesenchymal behaviours such as cell migration. However it is becoming increasingly clear that fibronectin is far more versatile than that (Armstrong and Armstrong, 2000). For example, during development pericellular fibronectin matrices play crucial roles in the polarisation and physical containment of epitheloid cells as well as in tissue compartmentalization (e.g. Marsden and DeSimone, 2001; Martins et al., 2009; Sakai et al., 2003; Trinh and Stainier, 2004; Zhou et al., 2008).

ECM receptors

Cells bind to the ECM through specific cell surface receptors, the best studied being dystroglycan, syndecans and integrins.

Dystroglycan (Fig. 1) is an ECM receptor, first identified in skeletal muscle. It is a transmembrane protein, composed of two subunits (α and β dystroglycan) synthesised from a single gene (*Dag1*). It forms a multimeric transmembrane molecular complex with other molecules which, through binding to laminin 211, is believed to confer stability to myotubes during muscle contraction (Moore and Winder, 2010). Defects in the glycosylation of the dystroglycan molecule, mutations in dystrophin, a member of the complex, or in the laminin α2 chain (*Lama2*) leads to various forms of muscular dystrophy (Huh et al., 2005; Moore and Winder, 2010). Apart from its major role in skeletal muscle, dystroglycan is also expressed in a variety of other tissues (epithelia, nervous system etc.) where it links the cytoskeleton to laminin and is thought to play a role in branching morphogenesis, cell polarisation and neuromuscular junction formation amongst others (Moore and Winder, 2010). The early lethality of *Dag1*-null embryos (E5.5) points to an essential role for dystroglycan in the formation and/or maintenance of a subset of embryonic basement membranes (Williamson et al., 1997).

Syndecans are membrane-intercalated proteoglycans containing a protein core bound to heparan sulphate and chondroitin sulphate glycosaminoglycan chains (Couchman, 2003; Rapraeger, 2001). There are four different syndecans in mammals (syndecans 1–4). Syndecans can bind ECM molecules directly, but they also bind growth factors through their glycosaminoglycan chains and they crosstalk with integrins both at the ligand binding and signalling level (Morgan et al., 2007). Syndecans have been implicated in adult myogenesis and muscle regeneration (Cornelison et al., 2004).

By far the most versatile and best studied of the ECM receptors are the integrins (Barczyk et al., 2010; Hynes, 1992; Legate et al., 2009; van der Flier and Sonnenberg, 2001). Integrins (Fig. 1) are heterodimeric glycoproteins composed of an α subunit non-covalently bound to a β subunit, both containing extracellular, transmembrane and cytoplasmic domains. In mammals, a total of 18 α subunits and 8 β subunits have been described to date. These combine into 24 different integrins (Table 2), each combination of α and β subunits forming a receptor for one or more ECM molecule. In some cases, integrins mediate cell–cell interactions and can even bind soluble molecules (Barczyk et al., 2010; van der Flier and Sonnenberg, 2001). At least 8 different α chains and two different β chains are expressed during skeletal muscle development (Table 3).

Integrins on the cell surface are allosteric proteins that exist in low, primed and high affinity states (Askari et al., 2009; Hynes, 2002). Their transition between those states is influenced by a variety of factors, such as ligand engagement and the binding of intracellular proteins to their cytoplasmic domains (Askari et al., 2009; Legate et al., 2009). Integrin–

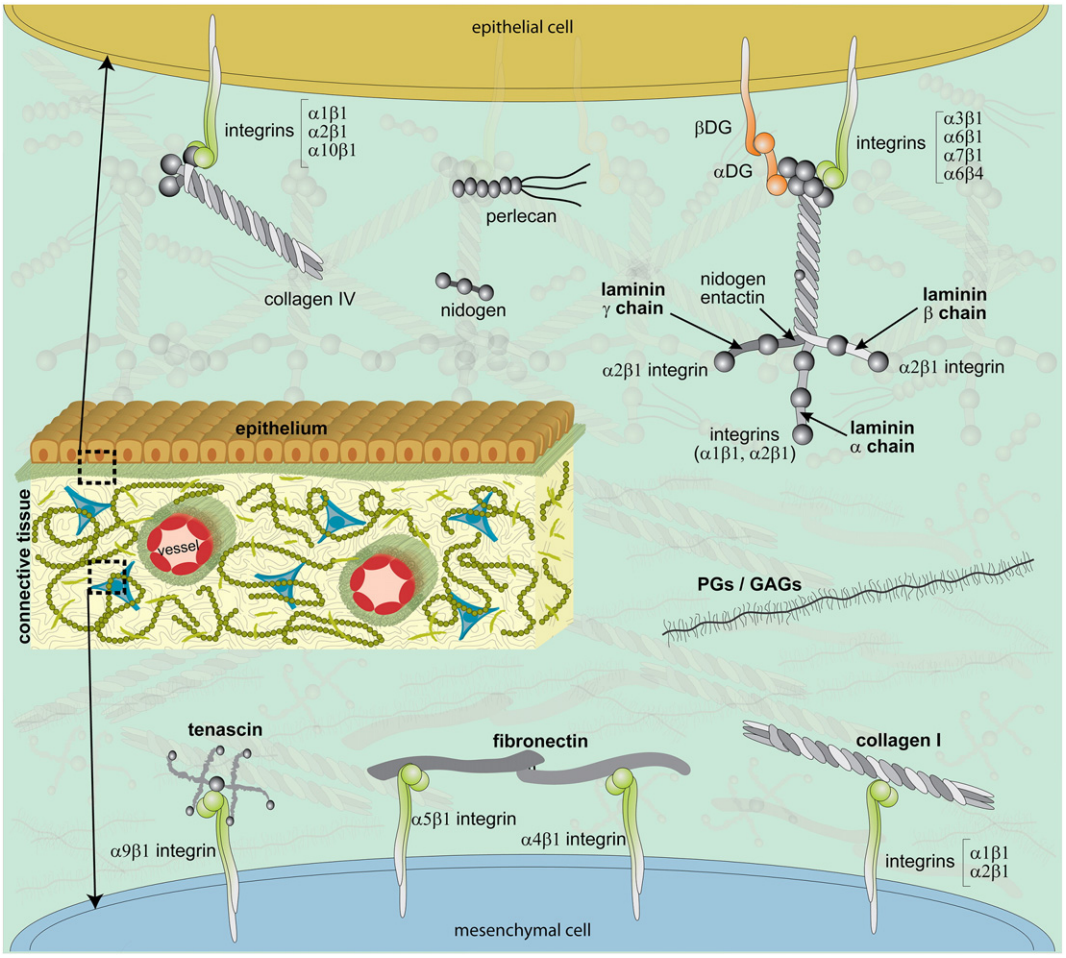

Fig. 1. The ECM and its relationship to cells. The ECM can be divided into interstitial and pericellular matrices. The interstitial matrix is illustrated in the central panel and contains interspersed mesenchymal cells (blue). The major ECM components are shown in the lower panel and are fibrillar collagens (typified as collagen type I), a variety of proteoglycans and glycosaminoglycans (PGs/GAGs), tenascin, fibronectin, amongst others. Of these, collagens, tenascin and fibronectin interact with cells through integrins. Pericellular matrices are illustrated in the central panel as the basement membranes (light green sheets) lining epithelia (brown cells) and blood vessels (red cells). The major basement membrane components are represented in the upper half of the panel, consisting of laminins, nidogen, perlecan and collagen type IV. Of these, collagen type IV binds to certain integrin receptors on the cell surface whilst laminin engages integrins and dystroglycan (DG). Laminins and collagen type IV assemble into a sheet-like network which also incorporates nidogen and perlecan.

ligand binding leads to conformational changes in the integrin which alter the affinity of its cytoplasmic domain for the proteins involved in numerous signalling pathways, whilst interaction of cytoplasmic proteins to their intracellular domain can modulate their ligand affinity (Askari et al., 2009; Danen and Sonnenberg, 2003; Hynes, 2002; Legate et al., 2009). Integrins also link to the actin (or intermediate filament) cytoskeleton through a variety of adaptor proteins, thus serving as a mechanosensor of the extracellular environment (Ingber, 2006; Schwartz and DeSimone, 2008). Which signalling pathways are activated upon ligand binding depends not only on the nature of the integrin and the ligand, but also on the cell type and cell context (Barczyk et al., 2010). Thus, apart from their direct signalling function, integrins can also feed into pathways activated by growth factors enhancing or inhibiting their effect (Comoglio et al., 2003; Danen and Sonnenberg, 2003; ffrench-Constant and Colognato, 2004; Walker et al., 2005). For example, integrin linked kinase (ILK) and its associated molecules (PINCHs and parvins) can inhibit GSK3β thus enhancing Wnt signalling, can activate Akt thus synergizing with PI3K signalling, and can modulate the activity of several Rho GTPases so affecting cell motility (Legate et al., 2006; Wickström et al., 2010). Another common integrin target, focal adhesion kinase (FAK) can activate MAP kinase and ERK and is a regulator of Rho GTPases (Schaller, 2010; Yee et al., 2008). Furthermore, the ECM can augment signalling downstream of growth factor receptors by concentrating signalling substrates in close proximity of these receptors and integrin-mediated adhesion to ECM can even cluster and activate growth factor receptors in the absence of their ligand (Comoglio et al., 2003; Danen and Sonnenberg, 2003; ffrench-Constant and Colognato, 2004; Walker et al., 2005).

In this way, integrin signalling has been shown to modulate proliferation, polarisation, migration, differentiation, survival and apoptosis. In agreement with a role in such a variety of cell behaviours, integrins are widely expressed, with practically all cells in the embryo and adult organism possessing their cell type- and/or developmental stage-specific integrin repertoire. Furthermore, integrins are stable cell surface proteins which can rapidly change their affinity for extracellular ligands and their connections to intracellular effectors (Askari et al., 2009; Hynes, 2002; Legate et al., 2009). Thus changes in integrin activity are not dependent on *de novo* transcription; in fact it is important for the developmental biologist to keep in mind that the absence of mRNA expression for a pair of integrin subunits in a tissue at a certain time does not necessarily mean that the protein is not present, since transcription might have occurred earlier in the life of that particular cell.

The extracellular matrix and skeletal muscle development

Somitogenesis and the establishment of the dermomyotome

All trunk and limb skeletal muscles of vertebrates are derived from the paraxial mesoderm, located on each side of the neural tube, extending

Table 1
The laminins.

Laminin	Old laminin nomenclature	Chain composition	Genes
Laminin 111	Laminin-1	α1β1γ1	*Lama1 Lamb1 Lamg1*
Laminin 121	Laminin-3	α1β2γ1	*Lama1 Lamb2 Lamg1*
Laminin 211	Laminin-2	α2β1γ1	*Lama2 Lamb1 Lamg1*
Laminin 221	Laminin-4	α2β2γ1	*Lama2 Lamb2 Lamg1*
Laminin 213	Laminin-12	α2β1γ3	*Lama2 Lamb1 Lamg3*
Laminin 212[a]	–	α2β1γ2	*Lama2 Lamb1 Lamg2*
Laminin 222[a]	–	α2β2γ2	*Lama2 Lamb2 Lamg2*
Laminin 311	Laminin-6	α3Aβ1γ1	*Lama3A Lamb1 Lamg1*
Laminin 321	Laminin-7	α3Aβ2γ1	*Lama3A Lamb2 Lamg1*
Laminin 332	Laminin-5	α3Aβ3γ2	*Lama3A Lamb3 Lamg2*
Laminin 3B32	Laminin-5B	α3Bβ3γ2	*Lama3B Lamb3 Lamg2*
Laminin 333	–	α3Aβ3γ3	*Lama3A Lamb3 Lamg3*
Laminin 411	Laminin-8	α4β1γ1	*Lama4 Lamb1 Lamg1*
Laminin 421	Laminin-9	α4β2γ1	*Lama4 Lamb2 Lamg1*
Laminin 423	Laminin-14	α4β2γ3	*Lama4 Lamb2 Lamg3*
Laminin 511	Laminin-10	α5β1γ1	*Lama5 Lamb1 Lamg1*
Laminin 521	Laminin-11	α5β2γ1	*Lama5 Lamb2 Lamg1*
Laminin 522[a]	–	α5β2γ2	*Lama5 Lamb2 Lamg2*
Laminin 523	Laminin-15	α5β2γ3	*Lama5 Lamb2 Lamg3*

Adapted from: Aumailley et al., 2005; Durbeej, 2010.
[a] Existence proposed.

Table 2
The integrins and their ligands.

Integrin	Genes	ECM ligand	Soluble ligand	Cell surface ligand
α1β1	*Itga1* *Itgb1*	Col I/IV/IX; Ln (α1, α2 chain)		Semaphorin 7A
α2β1	*Itga2* *Itgb1*	Col I/IV/IX; Ln (α1, α2, α5 chain)		E-cadherin, endorepillin
α3β1	*Itga3* *Itgb1*	Ln (α3, α5 chain); Tsp		
α4β1	*Itga4* *Itgb1*	Fn	pp-vWF;tTG; FXIII	VCAM-1
α4β7	*Itga4* *Itgb7*	Fn		VCAM-1; MadCAM
α5β1	*Itga5* *Itgb1*	Fn	tTG; endostatin	ADAM-15
α6β1	*Itga6* *Itgb1*	Ln (α1, α2, α3, α4, α5 chain)		ADAM-2,9
α6β4	*Itga6* *Itgb4*	Ln (α3, α5 chain)		
α7β1	*Itga7* *Itgb1*	Ln (α1, α2, α4, α5 chain)		
α8β1	*Itga8* *Itgb1*	Fn; Vn; Nn; Tn		
α9β1	*Itga9* *Itgb1*	Tn; Col I; Opn; Ln (α1 chain)	pp-vWF; tTG; FXIII; NGF; VEGF; angiostatin	VCAM-1; ADAM-12,-15
α10β1	*Itga10* *Itgb1*	Col II/IV/VI/IX		
α11β1	*Itga11* *Itgb1*	Col I/IV/IX		
αvβ1	*Itgav* *Itgb1*	Fn; Vn; Opn		
αvβ3	*Itgav* *Itgb3*	Vn, Fn, Tn, Ln (α1 chain); Opn	Fg; tumstatin	ADAM-15, −23
αvβ5	*Itgav* *Itgb5*	Vn; Opn	TGFβ-LAP; endostatin	
αvβ6	*Itgav* *Itgb6*	Fn; Tn	TGFβ-LAP	
αvβ8	*Itgav* *Itgb8*	Vn; Col IV; Fn; Ln (α1 chain)	TGFβ-LAP	
αIIbβ3	*ItgaIIb* *Itgb3*	Fn; Vn	Fg; vWF	
αLβ2	*Itgal* *Itgb2*			ICAM-1,2,3,5
αMβ2	*Itgam* *Itgb2*		Fg; iC3b; FX	ICAM-1; VCAM-1
αXβ2	*Itgax* *Itgb2*		Fg; iC3b	
αDβ2	*Itgad* *Itgb2*			ICAM-3; VCAM-1
αEβ7	*Itgae* *Itgb7*			E-cadherin

Abbreviations: ADAM, a disintegrin and metalloprotease; Col, collagen; Fg, fibrinogen; Fn, fibronectin; FX; coagulation factor X; FXII, coagulation factor XIII; iC3b, inactivated complement component C3b; ICAM, Intra-Cellular Adhesion Molecule; Ln, laminin; MadCAM, mucosal addressin cell adhesion molecules; Nn, nephronectin; Opn, osteopontin; pp-vWF, prepro-von Willebrand Factor; TGFβ-LAP, Transforming Growing Factor β latency-associated peptide; Tn, tenascin; Tsp, thrombospondin; tTG, tissue transglutaminase; VCAM, Vascular Cell Adhesion Molecule; Vn, vitronectin; vWF, von Willebrand Factor.
Table based on: van der Flier and Sonnenberg, 2001; Barczyk et al., 2010; Durbeej, 2010, with further details from Hu et al., 1995; Venstrom and Reichardt, 1995; Staniszewska et al., 2008.

along the axis of the embryo (Fig. 2). The paraxial mesoderm is organised in segments, the somites (Fig. 2A), which are formed in a sequential pattern all along the rostro-caudal axis (Kalcheim and Ben-Yair, 2005). Soon after their formation, the somites separate into the ventral sclerotome and the dorsal dermomyotome and subsequently some dermomyotomal cells give rise to the myotome (Fig. 2B). The sclerotome gives rise to the axial skeleton and the dermomyotome to all trunk, limb and a few head muscles, as well as some dermal, endothelial, smooth muscle and brown fat precursors (Buckingham, 2006; Kalcheim and Ben-Yair, 2005; Scaal and Christ, 2004; Seale et al., 2008). Finally, a population of cells (the so-called syndetome), located at the rostral and caudal interface between sclerotome and myotome (Fig. 2B), gives rise to the tendons linking muscles to the axial skeleton (Brent et al., 2003).

Although the division of the paraxial mesoderm into somites is not necessary for the differentiation of its different derivatives, the formation of segmentally organised epithelial somites is absolutely essential for the proper organisation of these derivatives, particularly the vertebral column (Burgess et al., 1996) and the hypaxial portion of the myotome (Wilson-Rawls et al., 1999). The fibronectin matrix, which normally surrounds the rostral presomitic mesoderm and early somites (Figs. 2A, 3A) (Duband et al., 1987; Ostrovsky et al., 1983, 1988) plays a crucial role in the process that converts the mesenchymal presomitic mesoderm into epithelial somites (George et al., 1993; Martins et al., 2009; Rifes et al., 2007). A fibronectin matrix is also assembled in the clefts between somites of the chick embryo (Martins et al., 2009; Rifes et al., 2007) and has proven to be essential to stabilise these clefts in zebrafish embryos (Koshida et al., 2005).

Soon after somites form, a basement membrane matrix containing laminin (Bajanca et al., 2004; Duband et al., 1987; Krotoski et al., 1986), collagen IV (Duband and Thiery, 1987; Leivo et al., 1980), nidogen/entactin (Zagris et al., 1993) and perlecan (Soulintzi and Zagris, 2007) is progressively laid down around the somite (Fig. 2A). Laminin 111 is probably the first constituent of this basement membrane since the gene encoding the laminin α1 chain, *Lama1*, is already transcribed in the presomitic mesoderm in the mouse (Anderson et al., 2009; Miner et al., 2004). All these basement membrane components remain present on the dermomyotome (Fig. 2B) after the sclerotomal cells de-epithelialise and disperse (Bajanca et al., 2004; Duband and Thiery, 1987; Krotoski et al., 1986; Zagris et al., 2000) and fibronectin remains closely associated with this basement membrane (Cachaço et al., 2005; Ostrovsky et al., 1988).

The dermomyotome as an epithelium of multipotent progenitors

The dermomyotome is a sheet-like epithelium with its basal side facing the ectoderm and curving into lips towards the sclerotome at its four sides (Figs. 2B, 3B,C). The dermomyotome is the precursor of all myogenic cells of the body and limbs, which are generated in a developmentally regulated and stepwise manner (Buckingham, 2006; Kalcheim and Ben-Yair, 2005; Scaal and Christ, 2004). The capacity of the dermomyotome to generate such a remarkable number of precursor cells must therefore depend on the tight regulation of mechanisms

Table 3
Integrin subunits expressed during mouse skeletal muscle development in vivo, their expression patterns in skeletal muscle and inactivation phenotypes. When the mouse expression pattern is unknown or incomplete, the chick expression pattern is presented. Abbreviations used: MPC = myogenic precursor cell.

Subunit	Expression pattern in muscle	Knock-out	References
α1	Mouse: myotome from E10.5; MPCs migrating to limbs; muscle masses at E11.5–E13.5	Viable and fertile; no phenotype in skeletal muscle	Gardner et al., 1996; Bajanca and Thorsteinsdóttir, 2002 Bajanca et al., 2004; Cachaço et al., 2005.
α3	Chick: adult myofibres	Die neonatally; abnormal development of kidneys, lung and skin; no phenotype in skeletal muscle	Bao et al., 1993; Kreidberg et al., 1996; DiPersio et al., 1997.
α4	Mouse: dermomyotome at E9.5; on differentiated myocytes epaxially, on hypaxial lip and young myotomal cells hypaxially; on both dermomyotome lips after dissociation of central dermyotome; E12.5 trunk and E12.5–14.5 forelimb muscle masses; primary and secondary myotubes; absent on secondary myoblasts	Die at E11–14; defects in placenta and heart; no skeletal muscle phenotype in chimaeras	Rosen et al., 1992; Yang et al., 1995; Yang et al., 1996b; Bajanca et al., 2004; Cachaço et al., 2005.
α5	Mouse:epithelial somites; differentiated myocytes in myotome; MPCs migrating to limbs; trunk and forelimb muscle masses at E12.5	Die at E10–11; defects in the posterior trunk, including impaired somite formation; chimaeras show muscle dystrophy	Yang et al., 1993; Goh et al., 1997; Taverna et al., 1998; Bajanca and Thorsteinsdóttir, 2002; Bajanca et al., 2004; Cachaço et al., 2005.
α6	Mouse: dermomyotome and myotome; E13.5–E14.5 trunk and limb muscles Chick: myotome; limb MPCs and myoblasts; primary myotubes	Die neonatally; severe skin blisters, absence of hemidesmosomes	Bronner-Fraser et al., 1992; Georges-Labouesse et al., 1996; Bajanca et al., 2004; Cachaço et al., 2005.
α7	Mouse: E10.5 myotome; E13.5 intercostal and pectoral muscles; myotendinous junctions	50% lethality at midgestation; 50% viable and fertile; progressive muscular dystrophy	Bao et al., 1993; Velling et al., 1996; Mayer et al., 1997; Bajanca et al., 2004; Cachaço et al., 2005.
α9	Mouse: E12.5 diaphragm and tongue, E14.5 myotubes	Die neonatally with respiratory failure	Wang et al., 1995; Huang et al., 2000.
αv	Mouse: E10.5 myotome; trunk and forelimb muscle masses at E12.5; E15.5 myotendinous junctions	80% lethality due to placental defects, 20% born with intracellebral haemorrhages	Hirsch et al., 1994; Bader et al., 1998; Schwander et al., 2003 Cachaço et al., 2005.
β1	Mouse: β1A present on all myogenic cells (including all muscle fibres) until E17.5, when it is progressively replaced by β1D	β1-null lethal at preimplantation; β1D-null viable; β1D knock-in lethal at midgestation (placental failure) or neonatally (reduced muscle mass); conditional β1-null (skeletal α-actin Cre) lethal neonatally (reduced muscle mass)	Fässler and Meyer, 1995; van der Flier et al., 1995, 1997; Zhidkova et al., 1995; Belkin et al., 1996; Baudoin et al., 1998; Cachaço et al., 2003; Schwander et al., 2003.
β3	Mouse: assumed to pair with αv at myotendinous junctions in E14.5 in conditional β1-null myotubes.	Viable and fertile; haemorrhage; placental defect; no phenotype in muscle	Hodivala-Dilke et al., 1999; Schwander et al., 2003

stimulating proliferation and inhibiting differentiation *versus* those promoting entry into the myogenic programme.

The dermomyotome epithelium grows progressively through symmetric cell divisions and cell flattening (Ben-Yair et al., 2003) and is characterised by the expression of the transcription factors Pax3 (Fig. 3B) and Pax7 which mark uncommitted cells (Buckingham and Relaix, 2007). Pax3 is required for the survival of cells in the hypaxial dermomyotome (Bajard et al., 2006; Borycki et al., 1999a) and Pax3/Pax7 expression is essential for the formation of all dermomyotome-derived myogenic precursors except those of the primary myotome (Relaix et al., 2005). Notch signalling has also been implicated in maintaining the undifferentiated state of the dermomyotome (Hirsinger et al., 2001).

Accumulating evidence also suggests that the epithelial state of the dermomyotome contributes to its capacity to maintain its uncommitted, proliferative state. The epithelial cells of the dermomyotome adhere to each other through N-CAM and N-cadherin, the latter being enriched in the apically localised adherens junctions (Duband et al., 1987) where it forms a complex with its intracellular partners β-catenin, plakoglobin (γ-catenin), p120 catenin and α-catenin (Meng and Takeichi, 2009). These proteins form the link between cadherins and the cytoskeleton and adherens junctions can function as a mechanosensor and signalling

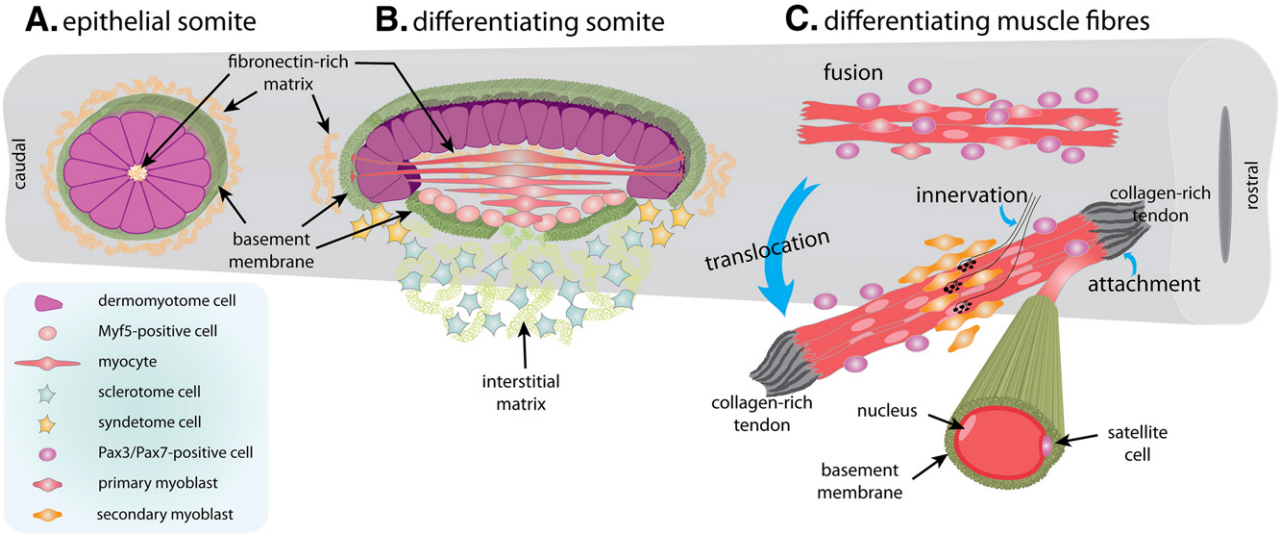

Fig. 2. Embryonic skeletal muscle development. A. Skeletal muscle is derived from somites which are epithelial balls of cells located on both sides of the neural tube. They are surrounded by a basement membrane and a fibrillar fibronectin matrix and also contain a loose fibronectin-rich matrix in their centre, the somitocoel. B. As the somite differentiates, the ventral part of each somite undergoes an epithelium-to-mesenchyme transition, giving rise to the mesenchymal sclerotome and its interstitial matrix. The dorsal part of the somite remains epithelial and is called the dermomyotome. Subsequently, Myf5-positive cells enter the myotomal space from the epaxial, hypaxial, rostral and caudal lips of the dermomyotome and organise the basement membrane that comes to separate the sclerotome from the myotome. These cells then migrate to the centre, where they start differentiating into elongated myocytes which eventually span the width of the segment. C. The dermomyotome dissociates, giving rise to Pax3/Pax7-positive cells whilst (in the trunk) myotomal myocytes translocate, changing orientation. Some Pax3/Pax7-positive cells differentiate into primary myoblasts which fuse with the preexisting myocytes, forming primary myotubes. As more and more primary myoblasts fuse with these primary myotubes (red), they elongate, assemble a laminin 211-containing basement membrane, get innervated (motor end plates represented as groups of black dots) and eventually attach to bone through tendons (grey) at their ends. Eventually, some Pax3/Pax7-positive cells, still present within the muscle masses, differentiate into secondary myoblasts. Secondary myoblasts adhere to primary myotubes near the innervation site and fuse with each other forming secondary myotubes along the primary myotubes. These progressively elongate, assemble their own basement membrane, get innervated and attach to tendons. Some Pax3/Pax7 cells remain within the muscle masses and give rise to satellite cells. These satellite cells become quiescent and reside on the surface of myofibres, under the basement membrane.

centre, maintaining the epithelial state (Heuberger and Birchmeier, 2010; Ingber, 2006). A fibronectin matrix is essential to induce the apical polarisation of N-cadherin in cells of the rostral presomitic mesoderm and to maintain the N-cadherin-containing adherens junctions in chick epithelial somites (Martins et al., 2009). Whether fibronectin still plays such a role at the dermomyotome stage or whether the laminin-containing basement membrane (Fig. 3C) has taken over this function has not yet been addressed. Adhesion through N-cadherin is essential to maintain the epithelial integrity of the dermomyotome in the quail as dermomyotomal cells electroporated with an N-cadherin lacking the extracellular domain deepithelialise, lose their basal laminin matrix and disperse into the subectodermal domain (Cinnamon et al., 2006). Canonical Wnt signalling through Wnt6, expressed in the ectoderm overlying the somites, is reported to be essential for the maintenance of the epithelial state of the dermomyotome in the chick (Linker et al., 2005), and canonical Wnt1 and Wnt3a signalling stimulates dermomyotome cell proliferation (Brauner et al., 2010; Galli et al., 2004). However, the key effector of canonical Wnt signalling, β-catenin, is also a major player in the maintenance of cadherin-based adhesions and recent studies have shown that the interdependence between these two pools of β-catenin is complex (Heuberger and Birchmeier, 2010). Thus it will be interesting to dissect out further the interplay between the adhesion and signalling functions of β-catenin in the maintenance of the dermomyotome.

The interaction of dermomyotomal cells with their basement membrane is also important for the maintenance of their undifferentiated state. The dermomyotomal basement membrane is composed of laminin 111 and 511 (Fig. 3C) as well as collagen type IV (Fig. 3D), perlecan and nidogen (Anderson et al., 2009; Bajanca et al., 2006) and the presence of the laminin receptor α6β1 integrin on the surface of dermomyotomal cells (Fig. 3B) indicates they bind to their basement membrane through this integrin (Bajanca et al., 2004). As described for other systems (Fuchs, 2007), positional cues not only from the N-cadherin-containing adherens junctions (Cinnamon et al., 2006; see above), but also from the basement membrane (Bajanca et al., 2006) may play critical roles in promoting symmetric cell divisions and the undifferentiated state of the dermomyotomal epithelium. In accordance with this hypothesis, blocking the binding between α6β1 integrin and laminin induces precocious myogenesis in the dermomyotome (Bajanca et al., 2006). Thus the experimental detachment of the dermomyotomal cells from their basement membrane appears to push the balance of signals promoting the undifferentiated state towards one that promotes differentiation. This indicates that for the epithelial cells of the dermomyotome, loss of contact to their basement membrane is one of the cues that drive their differentiation (also see The initiation of epaxial myotome formation section).

Bmp4, expressed in the dorsal neural tube and lateral mesoderm, has also been implicated as a repressor of precocious myogenesis in the dermomyotome (Hirsinger et al., 1997; Linker et al., 2003; Patterson et al., 2010; Reshef et al., 1998). Interestingly, during pancreatic development, Bmp-4 and −6 signalling and laminin 111-induced signalling through α6β1 and dystroglycan synergise to maintain the proliferative and undifferentiated state of pancreatic β-cell precursors (Jiang and Harrison, 2005). Whether a similar collaboration between Bmps and laminin occurs in the dermomyotome is not known, but studies in zebrafish indicate that extrinsic factors other than Bmps contribute in a decisive way towards maintaining the non-differentiated state of cells in the dermomyotome (Patterson et al., 2010). Altogether, these observations suggest that the epithelial state, including its characteristic pattern of cell–cell and cell–ECM engagement, collaborates and/or interacts with pathways activated by Notch, Wnts and Bmps, in order to repress precocious myogenesis in the dermomyotome.

The initiation of epaxial myotome formation

The first cells of the myotome originate from the epaxial lip of the dermomyotome (Fig. 3B,F; termed dorso-medial lip in avians) and give

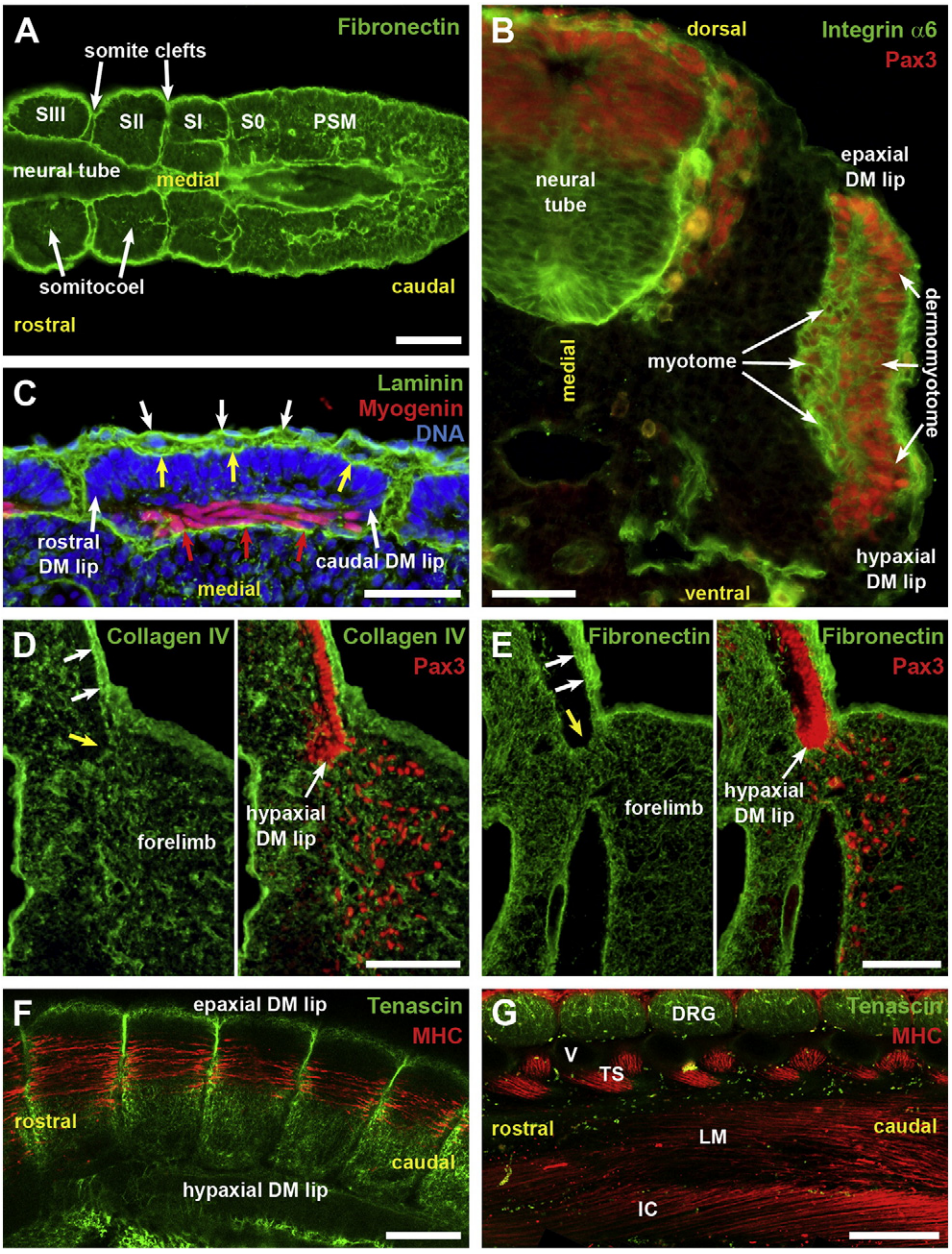

Fig. 3. Potential relationships between myogenic cells and the ECM. A. Longitudinal section of the tail of an E11.5 mouse embryo labelled for fibronectin. A fibronectin matrix surrounds the presomitic mesoderm (PSM) and epithelial somites and some immunoreactivity is also found in the somitocoel. The forming somite is termed S0, the most recently formed somite is SI and so forth. B. Transverse section of a caudal somite of an E11.5 mouse embryo labelled for integrin α6 (green) which is present in the myotome, dermomyotome, neural tube, blood vessels, notochord and ectoderm. Pax3 (red) marks the cells of the dermomyotome and the dorsal neural tube. Staining of blood cells is non-specific. C. Longitudinal section of the epaxial region of a dermomyotome/myotome of an E10.0 mouse embryo showing three distinct laminin (green)-containing basement membranes: the one lining the dermomyome basally (yellow arrows), the one separating the myogenin-positive (red) myotome from the sclerotome (red arrows) and the one lining the ectoderm (white arrows). D–E. Transverse sections showing delamination and initial migration of Pax3-positive (red) forelimb MPCs in an E10.0 mouse embryo. D. Collagen IV (green) lines the dermomyotome (white arrows), somatopleura, ectoderm, endoderm and blood vessels; note discontinuous staining at hypaxial lip (yellow arrow). E. A fibrillar fibronectin matrix (green) fills the mesenchymal space and MPCs invade this matrix during delamination (yellow arrow) and migration. F–G. Sagittal confocal sections of rat embryos at thorax level. F. E12.5 rat embryo. Segmented epaxial myotomes are forming with myocytes (red), parallel to the axis; segments are separated by a thick ECM (green). G. E15.5 rat embryo. Epaxial myocytes (red) have changed their orientation and length to form the complex epaxial muscle masses and the ECM (green) has been re-organised. PSM = presomitic mesoderm; DM = dermomyotome; MHC = myosin heavy chain; V = vertebra; DRG = dorsal root ganglia; TS = transverso-spinalis muscles; LM = longissimus muscle; IC = iliocostalis muscle. Scale bar: 50 μm (C); 100 μm (A, B, D, E, F); 250 μm (G). For methods see Bajanca et al. (2006) and Deries et al. (2010).

rise to the epaxial myotome (Denetclaw et al., 1997; Gros et al., 2004; Kahane et al., 1998; Patapoutian et al., 1995; Venters et al., 1999). Myogenesis is induced through the activation of the myogenic regulatory factors (MRFs) which commit cells to the myogenic lineage (Myf5, Mrf4, and MyoD) and then activate their differentiation programme (myogenin, MyoD, and Mrf4) (Buckingham, 2006). Wnt and Shh signalling have been shown to induce the expression of Myf5 in the epaxial lip, which is then followed by the translocation of the expressing cells to the underlying myotome and their subsequent differentiation into elongated myocytes (Bothe et al., 2007; Buckingham, 2006). Although low levels of Myf5 expression are already present in the rostral presomitic mesoderm and epithelial somites (Linker et al., 2003), sustained epaxial Myf5 expression requires Wnt1 (and/or Wnt3a) from the dorsal neural tube, Shh from the notochord/floorplate and inhibition of Bmp signalling through noggin expression (Amthor et al., 1999; Borycki et al., 1999b; Hirsinger et al., 1997; Münsterberg and Lassar, 1995). In the mouse,

canonical Wnt signalling synergizes with Shh signalling through Gli2/Gli3 to promote Myf5 expression (Borello et al., 2006; McDermott et al., 2005) and, in birds, Shh signalling has been shown to potentiate Wnt1 signalling by inducing *QSulf1*, a gene encoding an extracellular sulphatase which releases heparan sulphate-bound Wnt1 leading to MyoD activation (Dhoot et al., 2001). Interestingly, in zebrafish, a laminin-dependent deposition of heparan sulphate proteoglycans protects the central myotome from Bmp signals (Dolez et al., 2011) but whether such a mechanism restricts Bmp signalling during epaxial myogenesis in higher vertebrates has not been addressed.

Recent evidence in chick suggests that the translocation of the differentiating cells at the epaxial lip is preceded by an asymmetric cell division, as measured by spindle orientation and the asymmetric distribution of Numb (Holowacz et al., 2006; Venters and Ordahl, 2005). As a consequence, one daughter cell enters the myotome, whilst the other continues to reside in the dermomyotome epithelium. Numb is an inhibitor of Notch-signalling (McGill and McGlade, 2003). It was logical to suggest that its presence in the epaxial lip might inhibit Notch signalling, permitting the activation of MRFs and the initiation of myogenic differentiation (Venters and Ordahl, 2005; Holowacz et al., 2006). However, no effect on Notch signalling was observed when Numb was overexpressed under the control of the Myf5 Early Epaxial Enhancer in the mouse (Jory et al., 2009). Rather, overexpression of Numb favoured symmetric over asymmetric cell divisions, resulting in more dermomyotomal precursors and consequently more dermal and myogenic cells (Jory et al., 2009). It is becoming increasingly clear that Numb has many functions which are unrelated to Notch (Gulino et al., 2010). One of these involves the endo- and exocytosis of cadherins, contributing to the renovation of adherens junctions (Rasin et al., 2007; Wang et al., 2009); another is the recycling of integrins, contributing to cell migration (Nishimura and Kaibuchi, 2007). Whether Numb displays those functions in the dermomyotome remains to be seen. Nevertheless, the observation that Numb immunoreactivity is enriched on the basal side of dermomyotomal cells (Holowacz et al., 2006; Venters and Ordahl, 2005) raises the interesting possibility that it may be involved in modulating adhesion to the basement membrane by endocytosis of the α6β1 integrin. If Numb plays such a role at the epaxial lip it could weaken the attachment of cells to the basement membrane, before they enter mitosis, thus promoting asymmetric cell division. In support of this hypothesis, delaminating epaxial lip cells in the *Myf5*$^{nlacZ/nlacZ}$ mutant do not have α6β1 integrin on their surface (Bajanca et al., 2006). This demonstrates that there is a mechanism to remove this integrin and that cell surface expression is only restored in the myogenic daughter cell when Myf5 is active. The question of what induces the increased frequency of asymmetric cell divisions at the epaxial lip remains. As mentioned above, Wnt and Shh signalling have been shown to induce the expression of Myf5, but whether they also promote pathways leading to an asymmetric cell division, has not been addressed.

Formation and role of the myotomal basement membrane

The myotomal basement membrane separates the forming myotome from the sclerotome (Figs. 2B, 3C) and starts being assembled soon after the first Myf5-expressing cells enter the myotomal area (Anderson et al., 2007; Bajanca et al., 2006; Tosney et al., 1994). It is important to note that the myotomal basement membrane is distinct from the basement membrane of the dermomyotome (Tosney et al., 1994) and that it is also different from the laminin-211-containing basement membrane that surrounds differentiated muscle cells (Fig. 2C), which, in the case of the mouse, only becomes clearly detectable in the E11.5 myotome (Cachaço et al., 2005).

A major function of the myotomal basement membrane is to create a barrier between the myotome and the sclerotome and promote the spatial restriction of myotomal cells within the myotomal area. When there is a defect in the deposition of the myotomal basement membrane, myotomal cells tend to spread medially. The absence of a continuous basement membrane and, consequently, medial spreading of myotomal cells or their precursors is observed in embryos lacking *Myf5*/*Mrf4* (*Myf5*$^{nlacZ/nlacZ}$; Tajbakhsh et al., 1996), *Shh* and *Gli2/3* (Anderson et al., 2009), *Paraxis* (Wilson-Rawls et al., 1999) and *Dmrt2* (Seo et al., 2006), suggesting that the products of these genes play a direct or indirect role in the organisation of this basement membrane.

Unlike fibronectin matrix assembly, cell surface receptors are not required for laminin assembly, since laminin molecules bind to each other through their short arms (Fig. 1) even in the absence of cells (Colognato and Yurchenco, 2000; LeBleu et al., 2007; Yurchenco et al., 2004). However, cell–laminin interactions are necessary to place basement membranes in close contact with cells, where they are physiologically relevant, and serve as nucleating points for laminin assembly (Colognato and Yurchenco, 2000; LeBleu et al., 2007; Thorsteinsdóttir, 1992). Thus cells do not drive, but they organise laminin assembly. Both dystroglycan and β1-integrins have been implicated in organising a laminin matrix but exactly what role each plays is a matter of debate. Although some studies suggest that dystroglycan is required to bind laminin on the cell surface and β1-integrins are necessary for *de novo* laminin organisation (Henry et al., 2001; Lohikangas et al., 2001; Raghavan et al., 2000), both *Dag1*-null and *Itgb1*-null embryonic stem cell-derived embryoid bodies assemble a laminin matrix (Li et al., 2002). On the other hand, *Itgb1*-null embryos are developmentally retarded by E4.5 and form no true basement membranes (Fässler and Meyer, 1995; Stephens et al., 1995) and *Dag1*-null embryos are morphologically abnormal from E6.5 onwards with disrupted laminin organisation in Reichert's membrane (Williamson et al., 1997). This indicates an essential role for both receptors in the formation of at least a subset of basement membranes.

As far as we know, α6β1 (Fig. 3B) is the only laminin-binding β1 integrin expressed by the early epaxial myogenic precursors (Bajanca et al., 2004) and these cells also express dystroglycan (Anderson et al., 2007). Epaxial MPCs in *Myf5*$^{nlacZ/nlacZ}$ embryos do not retain the α6β1 integrin on their cell surface as they enter the myotome (Bajanca et al., 2006). Thus, *Myf5* expression is essential to maintain the α6β1 integrin on early epaxial myogenic cells. Furthermore, although laminin and other basement membrane molecules are detected in the myotomal area of *Myf5*$^{nlacZ/nlacZ}$ embryos, they do not organise into a continuous matrix (Bajanca et al., 2006). Consequently, the myogenic cells spread medially (Bajanca et al., 2006; Tajbakhsh et al., 1996). Myf5 therefore appears to play an essential, but indirect, role in myotomal basement membrane assembly by maintaining the α6β1 integrin on the cell surface. This integrin then mediates the organisation of the myotomal basement membrane into a barrier, retaining cells within the myotomal compartment (Bajanca et al., 2006). Whether dystroglycan is also required for the assembly of this basement membrane is currently not known.

Sonic hedgehog signalling is also essential for the assembly of the myotomal basement membrane. The expression of *Lama1* in epithelial somites and sclerotome depends on Shh signalling and when laminin 111 is absent, the basement membrane does not form, even though laminin 511 is present and MPCs express both dystroglycan and the α6β1 integrin (Anderson et al., 2009). Thus, as previously observed for Reichert's membrane (Miner et al., 2004), laminin 511 cannot substitute for laminin 111 in the formation of the myotomal basement membrane (Anderson et al., 2009). This is in contrast to its ability to partially compensate for the absence of laminin 111 in the basement membrane of the embryonic ectoderm/epiblast (Miner et al., 2004). It will be interesting to see in what way these situations differ, to understand why laminin 511 sometimes compensates for the absence of laminin 111 and sometimes not.

Paraxis (Wilson-Rawls et al., 1999) and *Dmrt2* (Seo et al., 2006) mutant embryos show a similar phenotype in the myotome, in that myotomal cells spread medially and elongated myocytes are disorganised and misaligned. In *Paraxis*-null embryos, mRNA expression for laminin was detected but protein distribution showed a disrupted

pattern (Wilson-Rawls et al., 1999). However, it is not clear which laminin isoform was present (laminin 111, laminin 511 or both). In *Dmrt2*-null embryos, the α1 chain of laminin 111 was undetectable both in the dermomyotome and in the myotome (Seo et al., 2006).

When cells enter the myotome they normally differentiate and organise into a parallel array of elongated myocytes (Fig. 2B). When laminin-α6β1 binding is blocked in explant cultures of wild-type embryos, the normal parallel alignment of elongating and elongated myocytes is disturbed, suggesting that α6β1 mediates the attachment of the myocyte tips to the myotomal laminin matrix (Bajanca et al., 2006). Both *Paraxis*- and *Dmrt2*-null embryos show severe defects in myocyte organisation and elongation (Wilson-Rawls et al., 1999; Seo et al., 2006), which is likely due to the absence of a normal myotomal basement membrane in both mutants. It remains to be seen whether Paraxis and Dmrt2 regulate the deposition of this basement membrane by controlling the production of laminin 111 (as in the case of Shh signalling) or whether they act through laminin receptors required for its assembly (as Myf5). The recent observation that Dmrt2 regulates *Myf5* expression raises the possibility that Dmrt2 affects laminin assembly in two ways: through laminin 111 production and by regulating *Myf5* and thus maintaining α6β1 integrin on myotomal cells (Sato et al., 2010). In accordance with this, overexpression of *Dmrt2* in the somite leads to enhanced laminin assembly near myotomal cells (Sato et al., 2010). Finally, in the chick embryo, Wnt11 and the PCP pathway have been shown to play crucial roles in the orientation of myotomal myocytes (Gros et al., 2009) but whether they do so directly or through influencing the organisation of the myotomal basement membrane has not been addressed.

After the initial stages of epaxial myotome formation, the myotome enters a new phase where it extends both in length (in the epaxial-hypaxial direction) and in width (in the medio-lateral direction). This growth is achieved by the entry of cells derived from all four dermomyotome lips (Buckingham, 2006; Kalcheim and Ben-Yair, 2005). The epaxial myotome grows in the dorsal direction by the addition of new cells from the growing epaxial dermomyotomal lip. At this stage, cells entering into the epaxial myotome are dependent on both Myf5 and Mrf4 expression to proceed with their myogenic programme (Kassar-Duchossoy et al., 2004). These cells express α6β1 on their surface and probably contribute to the growth of the myotomal basement membrane in a dorso-medial direction, since blocking α6β1-laminin binding in E9.5 embryo explants leads to discontinuities in the laminin matrix at the epaxial lip (Bajanca et al., 2006).

The myotome also grows in width by addition of cells coming from the rostral and caudal lips of the dermomyotome (Gros et al., 2004; Venters et al., 1999). These cells enter the myotome medially, between the pre-existing myocytes and the myotomal basement membrane (Fig. 2B), and the myotomal basement membrane probably acts as a substrate for cell migration mediated by α6β1 (Bajanca et al., 2006) and/or dystroglycan (Anderson et al., 2007). Interestingly, unlike α6β1, dystroglycan is enriched at the side where cells are in direct contact with the myotomal laminin matrix (Anderson et al., 2007), suggesting a different role from that of α6β1. Cells from rostral and caudal lips migrate in a caudal and rostral direction, respectively, until they reach midway between those lips (Fig. 2B). There they upregulate myogenin and start elongating, resulting in the characteristic V-shape of the myotome in the latero-medial direction (Venters et al., 1999). The tip of the V is an area where laminin immunostaining is discontinuous (Fig. 2B), indicating that laminin is being assembled there to account for the medial growth of the myotome (Bajanca et al., 2006). Furthermore, since myocyte elongation is dependent on contact between the myocyte tips and the laminin-containing myotomal basement membrane (Bajanca et al., 2006), one can hypothesise that elongation of the myotomal myocytes is facilitated by the rostro-caudal displacement of the basement membrane (Fig. 2B).

As the myocytes elongate, they also start expressing the α4β1 and α5β1 integrins and when they reach full length, their rostral and caudal ends cross the dermomyotome epithelium and appear to attach to the fibronectin-rich extracellular matrix present between segments (Fig. 2B) (Bajanca et al., 2004). It is thus plausible that α4β1 and/or α5β1 integrins and fibronectin play a role in reinforcing myocyte–ECM interactions at those intersegmental sites. Support for this hypothesis comes from studies in zebrafish, where knock-down of the two fibronectin genes (*fn1*+*fn3*) results in a perturbation of myotome boundaries which leads to disorganised slow myofibres and formation of abnormally long fast myofibres (Snow et al., 2008).

Finally, in the mouse embryo MyoD expression comes up relatively late, thus marking a different population than in the chick, where it is expressed at the onset of myogenesis in the somite (Buckingham et al., 1992). MyoD is first detected epaxially at around E10.5 and MyoD-positive elongated myocytes upregulate the laminin receptor α7β1, and, concomitantly, α6β1 expression in the myotome becomes considerable weaker (Bajanca et al., 2004). This coincides with the deposition of laminin 211 within the myotome (Cachaço et al., 2005) and thus indicates a shift in the adhesion of myocytes to the basement membrane separating the myotome from the sclerotome, to the adhesion to a pericellular laminin 211 matrix.

Early steps of hypaxial myogenesis

Hypaxial myogenesis involves two developmental programmes or a combination of these two programmes. The first one involves the ventral translocation of the hypaxial dermomyotomal lip and the hypaxial myotome into the somatopleure, giving rise to the prevertebral, intercostal and abdominal muscles, whereas the second programme involves the delamination of myogenic precursor cells (MPCs) from the hypaxial dermomyotome and their long-range migration to their target sites, giving rise to the muscles of the limbs, diaphragm and tongue (Bothe et al., 2007; Evans et al., 2006). Interestingly, certain muscle groups have adopted a combination of these two ways for translocation, as in the case of perineal muscles, where non-differentiated precursors migrate from the lateral dermomyotome into the hindlimb, upregulate MyoD and then translocate back into the trunk, towards the cloaca, where they adopt their final organisation (Evans et al., 2006; Valasek et al., 2005). Very little is known about what cell–ECM interactions occur during these developmental programmes.

The hypaxial myotome arises from the ventro-lateral aspect of the dermomyotome (Christ et al., 1983; Cinnamon et al., 1999; Denetclaw and Ordahl, 2000; Gros et al., 2004; Huang and Christ, 2000). The signalling pathways that induce hypaxial myotome formation are different from the ones seen epaxially in that Wnt7a from ectoderm signals non-canonically through PKC, leading to increased transcriptional activity of Pax3, and thereby activation of MyoD (Brunelli et al., 2007). As the hypaxial lip of the dermomyotome extends into the somatopleure it initially maintains its epithelial character and high levels of proliferation, suggesting that it is continuously providing new cells to the growing hypaxial myotome (Christ et al., 1983). Paraxis has been shown to contribute towards the maintenance of this epithelial and proliferative state of the hypaxial dermomyotome (Wilson-Rawls et al., 1999) and elevated Pax3 expression promotes its survival (Borycki et al., 1999a). However, as the ventro-lateral extension progresses, the hypaxial lip eventually dissociates giving rise to the muscle precursors of the abdominal musculature (Christ et al., 1983).

During early stages of hypaxial myotome development, laminin immunoreactivity is weaker and less continuous in the hypaxial myotome when compared to its epaxial counterpart (Bajanca et al., 2004; Cachaço et al., 2005) and the α6β1 integrin is only weakly expressed in the hypaxial lineage (Bajanca et al., 2004). In contrast, the fibronectin and VCAM1 receptor, α4β1 integrin is strongly

expressed by cells in the hypaxial lip of the dermomyotome as well as by early muscle precursors entering the hypaxial myotome (Bajanca et al., 2004). This is different from the situation epaxially where α4β1 is only present on differentiated myocytes (Bajanca et al., 2004) and suggests a difference in the cellular adhesion properties employed in early differentiation of the epaxial *versus* hypaxial lineage (Bajanca et al., 2004). It also suggests that α4β1-fibronectin (and/or α4β1-VCAM1) interactions may play a role in the ventral expansion of the hypaxial dermomyotome and myotome. This hypothesis is supported by the observation that implantation of hybridoma cells expressing an anti-β1 integrin antibody in the chick embryo disturbs the ventral growth of the hypaxial myotome (Jaffredo et al., 1988).

As mentioned above, at certain axial levels the hypaxial dermomyotomal lip gives rise to MPCs that migrate away from the dermomyotome, forming muscles (e.g. limb muscles, diaphragm, and tongue) at distant sites (Buckingham et al., 2003; Christ and Brand-Saberi, 2002; Francis-West et al., 2003). This event occurs earlier than the development of the hypaxial myotome described above. A key step in inducing delamination and migration at these levels is activation of the tyrosine kinase receptor Met (*c-met*) in the hypaxial dermomyotomal lip (Bladt et al., 1995; Heymann et al., 1996). *c-met* expression is induced hypaxially by Pax3 in all somites (Epstein et al., 1996; Relaix et al., 2003; Yang et al., 1996a), but Met signalling is only activated at the levels where migrating MPCs normally arise because the Met ligand, scatter factor/hepatocyte growth factor (SF/HGF), is secreted by the mesenchyme at those axial levels (Bladt et al., 1995; Brand-Saberi et al., 1996a; Dietrich et al., 1999; Heymann et al., 1996). Thus it is believed that Met signalling in response to SF/HGF engagement causes the epithelium-to-mesenchyme transition and subsequent migration. The effects of HGF/Met signalling in epithelial carcinomas are well known. Here, Met signalling generally downregulates E-cadherin expression either directly or indirectly (e.g. through Snail activation) leading to the dissociation of adherens junctions followed by induction of the expression of matrix metalloproteases (MMPs), such as MMP-2 and MMP-9, which degrade collagen type IV of basement membranes (Desiderio, 2007). Met signalling also activates focal adhesion kinase (FAK) which leads to changes in integrin function and modifications of the cytoskeleton, compatible with the migration phenotype (Desiderio, 2007). Although very little is known about how Met signalling affects these processes during delamination from the hypaxial dermomyotome, expression of *Itga6* mRNA, encoding the α6 integrin subunit of the α6β1 laminin receptor, is downregulated in limb level dermomyotomes precisely when MPCs are delaminating (Bajanca and Thorsteinsdóttir, 2002). Furthermore, as Pax3-positive MPCs exit and migrate away from the hypaxial lip, laminin and collagen IV lining the basal side of the hypaxial dermomyotome is discontinuous (Fig. 3D), indicating that not only are cells detaching from the basement membrane but this matrix is also being degraded. Interestingly, experimentally increasing Pax3 expression, as observed in *Pax3*$^{PAX3\text{-}FKHR\text{-}IRESnLacZ/+}$ embryos, results in increased Met activation in all somites, leading to ectopic disruption of the laminin-containing basement membrane of the hypaxial dermomyotome and cell delamination in thoracic somites (Relaix et al., 2006). Thus together these observations point to a link between Met signalling and basement membrane disengagement and degradation, leading to MPC delamination and cell motility.

Very little is known about which cell–ECM interactions are involved in MPC migration to the limbs and other distant sites. The homeobox transcription factor *Lbx1* is required for migration of MPCs into the hindlimb and regions of the forelimb, whilst playing a less important role in the movement of the MPCs of the hypoglossal chord and the diaphragm (Brohmann et al., 2000; Gross et al., 2000; Schäfer and Braun, 1999). Lbx1-positive MPCs express the cytokine receptor CXCR4 during their migration, responding chemotactically to the ligand stromal-derived factor 1 (SDF1) secreted by the mesenchyme (Vasyutina et al., 2005). CXCR4-signalling plays a crucial role in the directional migration of several embryonic cell populations (Raz and Mahabaleshwar, 2009). For example, CXCR4 signalling stabilises cell protusions on fibronectin in migrating neural crest cells from *Xenopus laevis* embryos (Theveneau et al., 2010). SDF-1/CXCR4 signalling has also been shown to promote cell anchorage to the ECM (Raz and Mahabaleshwar, 2009). In fact, high SDF-1 concentrations induce a CXCR4-dependent attachment of zebrafish endodermal cells to fibronectin through α5β1 and/or αvβ1 integrins (Nair and Schilling, 2008). CXCR4 signalling has been extensively studied in the hematopoietic system and in carcinomas where it is reported to increase expression of α4, α5, β1 and β3 integrin subunits, as well as FAK activity, which indicates it may act by modulating fibronectin receptors (Jones et al., 2007; Sanz-Rodríguez et al., 2001). Thus CXCR4 signalling promotes cell–ECM attachment in a variety of ways and it is tempting to suggest that it may control cell–ECM interactions during the migration of MPCs to the limb bud and possibly also their anchorage to the ECM when they reach their target sites. Migrating limb MPCs express *Itga5* and *Itga1* mRNA, whereas no signal is detected for *Itga4*, *Itga6* and *Itgav* (Bajanca and Thorsteinsdóttir, 2002). However, as mentioned earlier, integrins are stable proteins and thus the absence of mRNA signal does not necessarily mean the absence of protein. Nevertheless, based on these mRNA expression data, it seems likely that migrating MPCs are in a position to bind fibronectin (via α5β1) and collagens I, IV and IX, laminin 111 and 211 (via α1β1). A fibronectin matrix is present in the whole mesenchyme from the earliest stages of limb development (Fig. 3D) (Cachaço et al., 2005; Kosher et al., 1982), collagen IV and laminin line the ectoderm and blood vessels growing into the limbs (Fig. 3D) (Cachaço et al., 2005; Godfrey and Gradall, 1998), whereas collagens I and IX only appear later in tendon and cartilage development (Birk et al., 1997; Ros et al., 1995; Savontaus et al., 1998). Injection of an antibody against the cell-binding domain of fibronectin blocks MPC migration into the limb bud in the chick (Brand-Saberi et al., 1993) providing direct functional evidence for a role for α5β1-fibronectin engagement in MPC migration. Limb MPC migration is, however, not affected in chimeric mouse embryos containing wild-type and *Itgb1*-null (Fässler and Meyer, 1995) or *Itga5*-null (Taverna et al., 1998) cells. However, since MPCs adhere to each other and to the limb mesenchyme through N-cadherin (Brand-Saberi et al., 1996b), it is possible that *Itgb1*- and *Itga5*-null MPCs are carried to the limbs by adhering to migrating wild-type cells (Fässler et al., 1996). Hyaluronan has also been implicated in promoting MPC migration into the limb bud (Krenn et al., 1991). Whether this is by providing a substrate for cells, harbouring factors stimulating cell migration and/or whether it acts by creating hydrated spaces through which cells can migrate (Spicer and Tien, 2004) remains to be determined. In agreement with the studies in chick, regeneration of amputated axolotl limbs involves downregulation of collagen type I and laminin in the blastema whilst fibronectin, hyaluronan and tenascin-C are upregulated and form a transition matrix which promotes the migration of myoblasts into the regenerating area (Calve et al., 2010). Whether tenascin-C influences MPC migration during limb development has not yet been addressed, but seems unlikely since it is not expressed early enough (Kardon, 1998). However, it is expressed by early tendon precursors (Kardon, 1998; Ros et al., 1995) and may influence muscle formation later on (see The translocation of the myotome section).

Dissociation of the dermomyotome

The epithelial structure of the dermomyotome is maintained as the different waves of myotomal cells and/or hypaxial migrating MPCs are generated from the four lips (see above). Eventually, however, the dermomyotomal epithelium dissociates through an epithelium-to-mesenchyme transition associated with an asymmetric cell division, giving rise to dermal and myogenic precursors (Ben-Yair and Kalcheim, 2005; Cinnamon et al., 2006) as well as progenitors of endothelia and smooth muscle (Buckingham, 2006; Kalcheim and Ben-Yair, 2005; Scaal

and Christ, 2004). The dissociation process starts in the central dermomyotome at E10.5 in the mouse and around HH18 in the chick/quail, and progressively spreads from the central domain towards the epaxial and hypaxial lips (Ben-Yair and Kalcheim, 2005; Gros et al., 2005; Kassar-Duchossoy et al., 2005; Relaix et al., 2005). These lips then stay epithelial for some time before they eventually disintegrate (Tajbakhsh and Buckingham, 2000; Venters and Ordahl, 2002). In the chick, disintegration of the dermomyotome occurs shortly after the down-regulation of *Wnt6* in the overlying ectoderm and the loss of *Paraxis* expression in the dermomyotome (Geetha-Loganathan et al., 2006; Linker et al., 2005; Marcelle et al., 2002; Rodríguez-Niedenführ et al., 2003). Furthermore, recent evidence, also from the chick embryo, points to a role of myotomal Fgfs in signalling to the dermomyotome promoting MAPK/ERK signalling and the upregulation of *Snail1* (Delfini et al., 2009). The Snail genes are well known regulators of cell–cell and cell–ECM adhesion and act by promoting the endocytosis and transcriptional repression of E-cadherin (Baum et al., 2008; Guarino et al., 2007; Wu and Zhou, 2010), the adherens junction component of epiblast and epithelia of ectodermal and endodermal origin. However, N-cadherin, rather than E-cadherin, is the component of the dermomyotomal adherens junctions (Duband et al., 1987; Marcelle et al., 2002) and N-cadherin remains expressed on the cells that colonise the myotome (Cinnamon et al., 2006; Delfini et al., 2009). It is thus unclear exactly how Snail1 acts in the dermomyotome. One possibility is that it alone, or in synergy with Fgf signalling, promotes the internalisation of the N-cadherin of adherens junctions, but that this N-cadherin is then rapidly reinserted into the membrane in the daughter cells that colonise the myotome.

Dermomyotome dissociation not only involves changes in cell–cell adhesion, it also coincides with a downregulation of *Itga6* mRNA in the central dermomyotome (Bajanca and Thorsteinsdóttir, 2002), the expression of the matrix metalloprotease MMP2 which cleaves collagen IV (Duong and Erickson, 2004) and the disappearance of the dermomyotomal basement membrane. Whether there is any connection between the inducers of dermomyotome dissociation discussed above (downregulation of *Wnt6*, Fgf signalling or upregulation of *Snail1*) and the observed downregulation of *Itga6* or the activation of MMP2 has not been addressed. However, Fgf signalling and Snail proteins have been reported to disrupt cell-basement membrane engagement, activate MMP genes and promote cell migration in other systems (Baum et al., 2008; Guarino et al., 2007; Wu and Zhou, 2010).

As mentioned above, the loss of the epithelial morphology of the dermomyotome is accompanied by an asymmetric cell division (Ben-Yair and Kalcheim, 2005). In the quail embryo, the apical daughter cells, which enter the myotome as muscle precursors (or give rise to endothelial or smooth muscle cells) retain the apical N-cadherin-rich domain, Pax3/Pax7 expression and express the Fgf receptor FREK (Cinnamon et al., 2006). The myotomal cells have N-cadherin on their cell surface (Inuzuka et al., 1991), so it is reasonable to propose that the Pax3/Pax7-positive precursors entering the myotome adhere to the myotomal cells through homophilic N-cadherin binding, ensuring they remain within the muscle masses (Deries et al., 2010). Fgfs secreted by the myotome may also engage the FREK receptor (Kahane et al., 2001) and stimulate the proliferation of the Pax3/Pax7 positive cells. *Pax3*/*Pax7* double mutants have a severe muscle deficit (Relaix et al., 2005) suggesting that sustained *Pax3*/*Pax7* expression is essential for the self-renewal as well as the survival of this late population of dermomyotome-derived cells. The basal daughter cells of the asymmetric cell division, on the other hand, do not retain significant amounts of N-cadherin, downregulate Pax3/Pax7, but retain the expression of the homeodomain transcription factor *Alx4* (Ben-Yair and Kalcheim, 2005; Cinnamon et al., 2006), which marks their dermal fate (Cheng et al., 2004). Furthermore, since basement membrane degradation exposes the dermomyotomal cells to the adjacent interstitial matrix, these basal daughter cells are in a position to engage this matrix through integrins, promoting their migration into the dermis.

As mentioned above, the epaxial and hypaxial dermomyotomal lips persist in an epithelial state much longer than the central dermomyotomal sheet (Tajbakhsh and Buckingham, 2000; Venters and Ordahl, 2002) and, interestingly, at E11.5 both of these epithelial tissues express mRNA for the α4 integrin subunit (Bajanca et al., 2004). Whether this integrin promotes their epithelial state or whether it acts later when the lips eventually de-epithelialise, remains to be determined. Very few studies have addressed the mechanism behind the persistence of these epithelia, but in the chick embryo, Wnt11 in the epaxial lip and ectodermal Wnt6 near the hypaxial lip have been implicated in promoting their epithelial state (Geetha-Loganathan et al., 2006).

The translocation of the myotome

After the dissociation of the dermomyotome sheet, the myotome enters a new phase of development. Each myotome, by definition, spans only a single somite segment and is initially composed of mononucleate cells (Fig. 3F). Cell fusion occurs at the later stages of myotome development (Fig. 2C) and binucleate cells and multinucleate myotubes are found within the mouse myotome at E11.5 (Ontell et al., 1995; Venters et al., 1999). Soon after, the segmented structure of the myotomes disappears, and they contribute to the early muscle masses of the multi-segmental muscles (Figs. 2C, 3G). This reorganisation event is comparatively sudden, occurring within 36 h in the epaxial region of the mouse (from E11.5 until E13.0), raising the question of how this transformation is achieved in such a short period of time.

It has recently been demonstrated that the mononucleated epaxial myotomal myocytes do not undergo apoptosis but rather participate in the formation of the axial deep back muscles (Deries et al., 2010). As the myocytes become multinucleated and elongate, they undergo a change of orientation (Fig. 2C). The dorsal-most myocytes start to tilt at their rostral tip. This tilting has two consequences, first the myocytes are no longer parallel to the embryonic axis, and second, a cleavage plane is created that separates the dorsal myocytes from the rest of the myotome. Later on, more cleavage planes are formed and the myocytes of the four new epaxial muscle masses are no longer parallel to the axis (Fig. 3G) (Deries et al., 2010). The morphogenesis of the epaxial muscles raises the question of the force that drives the myotome to change into the complex axial muscles.

Progenitors of the connective tissue of muscles and of tendons expressing the transcription factor scleraxis (Pryce et al., 2007) are first only in the syndetome, but then they spread within the muscle mass (Deries et al., 2010). As myocytes move to create the future epaxial muscles, the connective tissue progenitors cluster at the end of the myocytes eventually creating the tendon anlagen (Deries et al., 2010). The connective tissue is therefore a good candidate to act as the motile force of the movement of the muscle cells. In this hypothesis, the communication of the myocytes with their environment is crucial, not only with the cells of the surrounding mesenchyme, but also with the ECM produced by this connective tissue. Interestingly, the expression pattern of integrins during the transformation of the myotome is conspicuous in the fact that the laminin receptors α6β1 and α7β1 integrins cease to be expressed after E11.5 to only reappear at E13.5 (Cachaço et al., 2005), specifically at the time when the translocation of the epaxial myocytes has terminated (Deries et al., 2010). Furthermore, the laminin-containing basement membrane separating the myotome from the sclerotome has disappeared by E11.5 and, although some laminin 211 is detected within the myotome, this staining is discontinuous (Cachaço et al., 2005). Thus, it is tempting to hypothesise that myocytes need to detach from their laminin anchor to be able to move.

In contrast to the laminin receptors, fibronectin receptors integrin subunits α4, α5 and αv are expressed during the whole transformation of the myotomes (Cachaço et al., 2005; Hirsch et al., 1994). A

prominent fibronectin matrix also stays present between and within the muscle masses during morphogenesis of epaxial muscles (Cachaço et al., 2005). The importance of fibronectin in the organisation of muscle masses has been pointed out by the knock-down of fibronectin genes in zebrafish which results in defects in the alignment and length of myocytes (Snow et al., 2008). Therefore, it is tempting to suggest that fibronectin may play a role in the reorganisation of the epaxial myocytes during their morphogenesis.

The glycoprotein tenascin-C is expressed in the sclerotome during neural crest migration (Riou et al., 1992). It is also known to be present in tendons, produced by fibroblasts (Chiquet-Ehrismann and Tucker, 2004). Whilst the cleavage planes are forming into myotomes to create the distinct epaxial muscle masses, tenascin-C remains present and even invades the newly formed gaps (Deries et al., 2010). αvβ3 is a receptor for tenascin-C and the αv integrin subunit is expressed during this process (Cachaço et al., 2005; Hirsch et al., 1994). It is enriched at myotendinous junctions later on (Schwander et al., 2003), suggesting a potential role for αvβ3-tenascin-C interactions in directing the events. This hypothesis contradicts the results obtained by inactivating tenascin-C in mice, as these mutants appear normal (Forsberg et al., 1996; Saga et al., 1992). However, a thorough investigation at the critical stages still needs to be done. Another hypothesis is that tenascin-C, which is part of the ECM present around the axial muscle masses, plays a role as a mechanical force and that the lack of tenascin-C is compensated by other ECM components such as fibronectin. Interstitial collagens could also be candidates to provide this mechanical force as they are present in the sclerotome at these critical stages (Ponticos et al., 2004). However, it is not clear whether they are then also expressed in the muscle masses.

The transformation of the hypaxial myotome into the definitive muscle masses has barely been investigated. Early researchers described interlimb hypaxial myotome blending and extending ventrally into the lateral body wall to create the different layers of abdominal muscles (Bardeen, 1900; Rizk and Adieb, 1982). Christ et al. (1983) confirmed the earliest studies by following the hypaxial dermomyotome and myotome with quail grafts in chick embryos. They described that the hypaxial lip of the dermomyotome together with the myotome extend in the lateral abdominal wall. As mentioned above, the dermomyotome undergoes an epithelium to mesenchyme transition whilst the myotomes continue to extend within the flank of the embryo and finally lose their segmented structure. The muscle mass then divides into the different layers of the abdominal muscles and they continue to extend together within the abdominal wall to reach their final position. As muscle masses segregate and myotubes change their orientation, the connective tissue invades the cleavage planes in between the layers of muscles. Cinnamon et al. (1999) used injection of fluorescent vital dyes to trace the final destiny of individual cells injected whilst in the early myotome at late developmental stages in the quail. They found marked cells in intercostal muscles, proving that hypaxial myotomal cells contributed to the formation of intercostal muscle fibres. Thus, the initial scaffold of intercostal muscles is directly formed from the hypaxial myotome *in situ*, showing that myotomal myocytes need to change their orientation and the hypaxial myotome needs to segregate to give rise to the three different layers of intercostal muscles.

The transformation of hypaxial myotome therefore seems to be of the same nature as that of the epaxial myotome. Since ECM components and their integrin receptors expression pattern appears to be the same in the dorsal and the ventral part of the trunk at this stage (Cachaço et al., 2005) it seems possible that the mechanisms involved in the transformation of both parts of the myotome are similar. However, subtle differences could be encountered in the different parts of the myotome because during earlier stages of myotome development the cell–ECM adhesion properties epaxially and hypaxially are different (Bajanca et al., 2004; also see above). A thorough investigation would be necessary to confirm and complete these theoretical models.

The formation of multinucleated myotubes

The next step in myogenesis is the differentiation of myogenic cells into fusion-competent myoblasts and the fusion of these cells into multinucleated myotubes, which then mature into muscle fibres (Biressi et al., 2007; Hauschka, 1994; Stockdale, 1992; Wigmore and Dunglison, 1998;). The early stage muscle masses give rise to three different populations of myoblasts: primary (also called embryonic), secondary (also called foetal) and adult (also called satellite cell) myoblasts (Biressi et al., 2007; Hauschka, 1994; Stockdale, 1992; Wigmore and Dunglison, 1998). The process by which myotubes are formed and grow is called primary or secondary myogenesis, depending on the embryonic stage at which it happens, and the types of myoblasts involved (Kelly and Zacks, 1969; Ontell and Kozeka, 1984; Ross et al., 1987). Primary myogenesis starts in the myotome of the mouse at E11.5 (Fig. 2C) and ends around E14.5. It involves the fusion of primary myoblasts to myotomal myocytes (or to each other), giving rise to binucleated myotubes, followed by the fusion of primary myoblasts to those myotubes (Biressi et al., 2007; Buckingham, 2001; Deries et al., 2010). Primary myogenesis in the limbs starts about half a day later and first involves the fusion of primary myoblasts to each other and then the fusion of myoblasts to those myotubes (Biressi et al., 2007; Buckingham, 2001; Christ and Brand-Saberi, 2002). Most importantly, primary myotubes set the orientation of all muscles and serve as a scaffold for the remaining stages of myogenesis (Hauschka, 1994). Secondary myogenesis starts at around E14.5 in the mouse when secondary myoblasts enter the myogenic programme and adhere to primary myotubes near innervations sites and, using the primary myotubes as a scaffold, fuse with each other giving rise to secondary myotubes (Fig. 2C) (Duxson et al., 1989; Harris et al., 1989). Notably, secondary myoblasts also fuse to the ends of primary myotubes, thus contributing to their growth (Zhang and McLennan, 1995). Here we will primarily focus on how cell–ECM interactions contribute to the formation of primary myotubes, the scaffold that sets the direction and organisation of the muscles to be.

To address how cell–ECM interactions contribute to myotube formation, focus has been turned to the role of β1 integrins. Since inactivation of *Itgb1* in the mouse leads to a very early lethality (Fässler and Meyer, 1995; Stephens et al., 1995), not allowing for the study of the role of β1 integrins in myogenesis, embryo chimaeras composed of wild-type and *Itgb1*-null cells were produced. These revealed normal myotubes composed of a mixture of wild-type and *Itgb1*-null nuclei suggesting that *Itgb1*-null myoblasts can fuse with wild-type cells (Fässler and Meyer, 1995), raising the question whether cell–ECM interactions through β1 integrins are necessary for myotube formation. However, the observation that ES-cell derived (i.e. primary) *Itgb1*-null myoblasts showed impaired fusion *in vitro*, whereas *Itgb1*-null secondary myoblasts isolated from late gestation wild-type/*Itgb1*-null chimaeras fused normally (Hirsch et al., 1998), gave the first indication that β1 integrins were important at least for primary myogenesis. Nevertheless, since *in vitro* culture conditions tend to be more permissive for fusion than the *in vivo* environment (Gullberg et al., 1998; McLennan and Koishi, 2002), further studies were necessary to confirm these results *in vivo*. This was addressed by using a mouse line where the striated muscle specific β1D splice variant, normally only expressed in skeletal muscle from E17.5 (Brancaccio et al., 1998; van der Flier et al., 1997), was knocked into the locus of the ubiquitously expressed β1A variant (Baudoin et al., 1998). β1D differs from β1A in its cytoplasmic domain and in its interaction with intracellular effectors such as talins, α-actinin, filamins (Belkin et al., 1997; Pfaff et al., 1998) and ILK (Hannigan et al., 1996), without any effect on ligand specificity (Belkin et al.,

1997). Analysis of embryos homozygous for the β1D knock-in allele revealed a reduction in skeletal muscle mass due to an impairment of primary myogenesis, evidenced by fewer and smaller primary myotubes (Cachaço et al., 2003). Interestingly, this effect was specific for primary myogenesis since secondary myogenesis proceeded normally until limited by the availability of primary myotubes (Cachaço et al., 2003). These results demonstrated that β1A is essential for the formation of primary myotubes and that β1D is unable to substitute for β1A in this process. These results also showed that either β1 integrins are dispensable for secondary myogenesis or their function is readily performed by β1D. Subsequently, conditional inactivation of *Itgb1* (i.e. both β1A and β1D) with a human skeletal α-actin Cre, also showed an impairment in primary myogenesis *in vivo* with the formation of fewer primary myotubes and an accumulation of unfused myoblasts (Schwander et al., 2003). Interestingly, secondary myogenesis was also impaired and an analysis of this process *in vitro*, revealed abnormalities in myoblast fusion, costamere structure and sarcomere maturation, which were not rescued by conditioned medium from wild-type cultures (Schwander et al., 2003). Thus, β1 integrins play an essential role in myotube formation both during primary and secondary myogenesis. Furthermore, the results of Cachaço et al. (2003) demonstrate that whereas β1A is essential for primary myogenesis, both β1A and β1D can mediate cell–ECM interactions during secondary myogenesis.

Although cell–ECM interactions through β1 integrins have been established as necessary for both primary and secondary myogenesis, it is still unclear exactly which ligands in the ECM are involved in these interactions. β1 pairs with 12 different αs, giving rise to integrins recognising at least 12 different ECM molecules, with considerable redundancy (see Table 2). However, some cues are available for primary myogenesis. The two major laminin-binding integrins of skeletal muscle (α6β1 and α7β1) are absent when primary myotubes are being formed and a continuous basement membrane is lacking in both limb and axial muscle masses (Cachaço et al., 2005; Chiu and Sanes, 1984; Godfrey and Gradall, 1998), arguing against a role for cell–laminin interactions in the events leading up to primary myoblast fusion. However, these integrins are reexpressed on myotubes after their formation, which correlates with the deposition of a laminin 211-containing basement membrane around the myotubes (Cachaço et al., 2005; Chiu and Sanes, 1984; Gullberg et al., 1998), suggesting a role in myotube maturation and/or stability. Of the integrin subunits expressed during primary myotube formation (α1, α4, α5 and αv), all but α1 are fibronectin receptors (see Table 2), and fibronectin is present within the early muscle masses at all stages of primary myogenesis (Cachaço et al., 2005). These observations suggest a potential role for cell–fibronectin interactions, possibly in cell alignment in preparation for fusion, as suggested by fibronectin knock-down in zebrafish (Snow et al., 2008). Inactivation of *Itga1* (Gardner et al., 1996) and *Itgav* (Bader et al., 1998) did not show any phenotype in skeletal muscle in the mouse and unfortunately, mouse embryos null for *Fn1* (George et al., 1993), *Itga4* (Yang et al., 1995, 1999) and *Itga5* (Yang et al., 1993, 1999) die too early to address this issue *in vivo*. Thus exactly what cell–ECM interactions regulate primary myotube formation in the embryo is still an open question.

It is well known that the connective tissue around the developing muscle masses of trunk and limbs plays an essential regulatory role in their differentiation, subdivision into separate muscle anlagens, and in the orientation of primary myotubes within those anlagens (Brent et al., 2003; Chevallier et al., 1977; Christ et al., 1977; Deries et al., 2010; Hurle et al., 1990; Kardon, 1998; Mathew et al., 2011; Shellswell et al., 1980; also see The translocation of the myotome section). For example, it was recently proved that fibroblasts of muscle connective tissue expressing the transcription factor Tcf4 are needed to regulate foetal myogenesis (Mathew et al., 2011). The inactivation of *Tcf4* leads to the reduction of the proportion of slow fibres and an increase of embryonic fibres in limb muscles. Moreover, the loss of Tcf4-expressing fibroblasts results in reduction of slow MHC. These results strongly suggest that muscle fibroblasts promote the maturation of myofibres. This regulation is both dependent and independent of Tcf4 and might act through the regulation of cell–ECM interactions. In fact, dynamic changes in ECM composition occur during these events (Fernandez et al., 1991; Olguin and Brandan, 2001) but much is yet to be known about the specific ECM molecules within the connective tissue and the cell–ECM interactions involved in the shaping of the early muscle masses (Edom-Vovard and Duprez, 2004; Evans et al., 2006). Nevertheless, it is clear that the failure of muscle fibres to connect to the ECM of developing tendons during development, leads to their apoptosis (Rodriguez-Guzman et al., 2007). The increased apoptosis seen in the muscle masses of β1D knock-in embryos (Cachaço et al., 2003) could be due to the failure of the short myofibres to establish tendon connections and cell detachment from tendons was indeed observed in muscles lacking β1 integrins (Schwander et al., 2003). Interestingly, activation and inhibition of retinoic acid signalling contributes to the shaping of muscles by influencing their attachment to the ECM, where retinoic acid signalling downregulates β1 integrins, thus causing myofibre detachment and apoptosis (Rodriguez-Guzman et al., 2007). The challenge ahead is to dissect out exactly what cell–ECM interactions are involved in these phenomena and to learn more about how the connective tissue ECM orients and shapes skeletal muscles.

Future perspectives

The ECM is now established as an essential player in skeletal muscle development. However, many gaps in our knowledge still remain. A fascinating area to address further is how cell–ECM interactions contribute towards regulating the precise equilibrium between self-renewing cells and the phased induction of myogenic commitment in the dermomyotome. Another area that merits further study is which ECM molecules and integrin receptors are involved in the translocation of MPCs and differentiated myocytes. In the case of MPCs, do they use multiple ECM molecules and multiple integrins or do they require a specific substrate on their migration path? In the case of the translocation of differentiated cells, do they actively migrate, or do they attach to key points in the ECM and become “displaced” to a different location by mechanical forces within the changing environment? Potential differences in the events leading up to the formation of primary *versus* secondary myotubes is also a fascinating subject to address. Primary myoblasts fuse in an environment composed of an interstitial ECM, whereas secondary myoblasts use primary myotubes (and potentially also their basement membrane) as a scaffold for their alignment and fusion. It will be interesting to dissect out how these distinct environments differ in terms of cell–ECM interactions and how each promotes myotube formation. Finally, although not the subject of this review, it is also urgent to understand how cell–ECM interactions contribute to adult myogenesis and muscle repair, which has clear applications for designing therapies for muscle dystrophies. Do cell–ECM interactions contribute to maintain muscle precursors within the muscle stem cell niche and how do those cell–ECM interactions change upon activation, proliferation and differentiation of the muscle satellite cells into fusion-competent myoblasts? Can we learn from the “dermomyotome niche” or are the programmes of the adult “muscle stem cell niche” different? Further investment into studying the ECM dimension of skeletal muscle development in the embryo will certainly provide a fuller understanding of embryonic myogenesis. The knowledge obtained can then be applied to the study of adult myogenesis and the treatment of muscle dystrophies.

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