Results and Problems in Cell Differentiation 56

Beate Brand-Saberi *Editor*

Vertebrate Myogenesis

Stem Cells and Precursors

Springer

Results and Problems in Cell Differentiation

Volume 56

Series Editors
Dietmar Richter
Henri Tiedge

More information about this series at
http://www.springer.com/series/400

Beate Brand-Saberi
Editor

Vertebrate Myogenesis

Stem Cells and Precursors

Springer

Editor
Beate Brand-Saberi
Abt. für Anatomie und Molekulare Embryologie
Ruhr-Universität Bochum Medizinische Fakultät
Bochum
Germany

Series Editors
Dietmar Richter
Center for Molecular Neurobiology
University Medical Center
Hamburg-Eppendorf (UKE)
University of Hamburg
Hamburg Germany

Henri Tiedge
The Robert F. Furchgott Center for Neural
and Behavioral Science
Department of Physiology and
Pharmacology
Department of Neurology
SUNY Health Science Center at Brooklyn
Brooklyn, NY, USA

ISSN 0080-1844 ISSN 1861-0412 (electronic)
ISBN 978-3-662-44607-2 ISBN 978-3-662-44608-9 (eBook)
DOI 10.1007/978-3-662-44608-9
Springer Heidelberg New York Dordrecht London

Library of Congress Control Number: 2014953240

© Springer-Verlag Berlin Heidelberg 2015
This work is subject to copyright. All rights are reserved by the Publisher, whether the whole or part of the material is concerned, specifically the rights of translation, reprinting, reuse of illustrations, recitation, broadcasting, reproduction on microfilms or in any other physical way, and transmission or information storage and retrieval, electronic adaptation, computer software, or by similar or dissimilar methodology now known or hereafter developed. Exempted from this legal reservation are brief excerpts in connection with reviews or scholarly analysis or material supplied specifically for the purpose of being entered and executed on a computer system, for exclusive use by the purchaser of the work. Duplication of this publication or parts thereof is permitted only under the provisions of the Copyright Law of the Publisher's location, in its current version, and permission for use must always be obtained from Springer. Permissions for use may be obtained through RightsLink at the Copyright Clearance Center. Violations are liable to prosecution under the respective Copyright Law.
The use of general descriptive names, registered names, trademarks, service marks, etc. in this publication does not imply, even in the absence of a specific statement, that such names are exempt from the relevant protective laws and regulations and therefore free for general use.
While the advice and information in this book are believed to be true and accurate at the date of publication, neither the authors nor the editors nor the publisher can accept any legal responsibility for any errors or omissions that may be made. The publisher makes no warranty, express or implied, with respect to the material contained herein.

Printed on acid-free paper

Springer is part of Springer Science+Business Media (www.springer.com)

Contents

Recruitment of Skeletal Muscle Progenitors to Secondary Sites: A Role for CXCR4/SDF-1 Signalling in Skeletal Muscle Development 1
Maryna Masyuk and Beate Brand-Saberi

Hypaxial Muscle: Controversial Classification and Controversial Data? 25
Karl R. Wotton, Frank R. Schubert, and Susanne Dietrich

Skeletal Myogenesis in the Zebrafish and Its Implications for Muscle Disease Modelling 49
David Gurevich, Ashley Siegel, and Peter D. Currie

Mechanisms of Myogenic Specification and Patterning 77
Mordechai Applebaum and Chaya Kalcheim

The Avian Embryo as a Model System for Skeletal Myogenesis 99
Claire E. Hirst and Christophe Marcelle

Head Muscle Development 123
Eldad Tzahor

The Lateral Plate Mesoderm: A Novel Source of Skeletal Muscle 143
Qin Pu, Ketan Patel, and Ruijin Huang

Regulation of Skeletal Muscle Development and Disease by microRNAs 165
Ning Liu and Rhonda Bassel-Duby

Adult Skeletal Muscle Stem Cells 191
Ramkumar Sambasivan and Shahragim Tajbakhsh

Dormancy and Quiescence of Skeletal Muscle Stem Cells 215
Pierre Rocheteau, Mathilde Vinet, and Fabrice Chretien

Index 237

Recruitment of Skeletal Muscle Progenitors to Secondary Sites: A Role for CXCR4/SDF-1 Signalling in Skeletal Muscle Development

Maryna Masyuk and Beate Brand-Saberi

Abstract During embryonic development, myogenesis occurs in different functional muscle groups at different time points depending on the availability of their final destinations. Primary trunk muscle consists of the intrinsic dorsal (M. erector spinae) and ventral (cervical, thoracic, abdominal) muscles. In contrast, secondary trunk muscles are established from progenitor cells that have migrated initially from the somites into the limb buds and thereafter returned to the trunk. Furthermore, craniofacial muscle constitutes a group that originates from four different sources and employs a different set of regulatory molecules. Development of muscle groups at a distance from their origins involves the maintenance of a pool of progenitor cells capable of proliferation and directed cell migration. We review here the data concerning somite-derived progenitor cell migration to the limbs and subsequent retrograde migration in the establishment of secondary trunk muscle in chicken and mouse. We review the function of SDF-1 and CXCR4 in the control of this process referring to our previous work in shoulder muscle and cloacal/perineal muscle development. Some human anatomical variations and malformations of secondary trunk muscles are discussed.

1 Introduction

The development of skeletal muscles is a highly regulated process in all classes of vertebrates. It involves a sensitive balance between commitment, differentiation, and proliferation not only at the sites of origin but also at destinations that become available during the course of development. The latter is a common scenario especially in higher vertebrates. During evolution, the scenario became more complex with the construction of increasingly efficient limbs and the acquisition of the head and neck. The emergence of all sites in the vertebrate body apart from the segmental dorsal (epaxial) muscle involves subsequent processes that require a redistribution of cells to locations where they undergo differentiation in later phases

M. Masyuk • B. Brand-Saberi (✉)
Department of Anatomy and Molecular Embryology, Ruhr-Universität Bochum,
Universitätsstraße 150, MA 5/161, 44801 Bochum, Germany
e-mail: Maryna.Masyuk@ruhr-uni-bochum.de; Beate.Brand-Saberi@rub.de

© Springer-Verlag Berlin Heidelberg 2015
B. Brand-Saberi (ed.), *Vertebrate Myogenesis*, Results and Problems in Cell
Differentiation 56, DOI 10.1007/978-3-662-44608-9_1

of development. This long-range redistribution involves cells of the hypaxial lineage, although the mechanisms may differ with regard to particular locations and muscle groups in the body. What is common to all of them is the fact that premature differentiation has to be delayed until the cells have settled in their final locations. This is due to migration and differentiation being mutually exclusive events in myogenesis. During ontogenesis, the ventrolateral body wall develops later than the dorsal body wall, and the hypaxial lineage populating these regions will only find their targets and have space to unfold subsequent to development of the epaxial lineage. In this way, mechanisms must have developed during evolution to keep the cells in a poised condition, while at the same time guidance cues must be provided for migratory routes to defined goals. In this article, we will address the orchestration of secondary muscle group development in mammals and avian species, focusing in particular on the migratory subpopulations. Investigations of the last decade have revealed that beside the role of Scatter Factor/Hepatocyte Growth Factor (SF/HGF) and its tyrosine kinase receptor Met (Bladt et al. 1995; Brand-Saberi et al. 1996a, b; Dietrich et al. 1999; Scaal et al. 1999), chemokines and their receptors are also involved in cell migration associated with myogenesis, in particular the cell migration of muscle progenitors to “secondary” sites of muscle formation (Yusuf et al. 2005; Rehimi et al. 2010). The CXC chemokine SDF-1 (stromal-cell-derived factor 1/CXCL12) and its G-protein coupled receptor CXCR4 are critical in numerous physiological and pathological processes, such as haematopoiesis (Zou et al. 1998), inflammatory response (Bleul et al. 1996a, b), and HIV-pathogenesis (Deng et al. 1996). Most significantly, this receptor–ligand pair has been shown to play a role in a number of migration processes during embryogenesis, such as migration of primordial germ cells towards the gonads (Doitsidou et al. 2002). In the developing nervous system, SDF-1 guides the migration of granular cells to their position in the cerebellum (Klein et al. 2001; Zou et al. 1998) and cells in the dentate gyrus of the hippocampus (Lu et al. 2002). Furthermore, CXCR4 signalling is active also in organ regeneration (Askari et al. 2003). Thus, it is not surprising, that the CXCR4/SDF-1 axis has also been implicated in cell migration during the development of skeletal musculature.

In this review article, we intend to provide an overview of the present research data concerning the role of the CXCR4/SDF-1 signalling during skeletal muscle development, with a particular focus on migrating myogenic precursor cells. Furthermore, we discuss different skeletal muscle groups, their developing modes, and molecular players controlling several phases of their formation.

2 The Somites as Turntables of Progenitor Cells

In vertebrates, the most significant and most investigated embryonic source of skeletal muscles and their progenitors are the somites. These structures develop from the paraxial mesoderm in a cranio-caudal direction and are segmentally arranged on both sides of the embryo lateral to the neural tube and the notochord

(reviewed by Aulehla and Pourquié 2006). Initially consisting of a pseudostratified epithelial layer containing a few mesenchymal somitocoel cells, the somites undergo a number of morphological changes, which lead to the formation of somitic compartments (Brand-Saberi et al. 1996a, b; Christ et al. 1972; Christ and Ordahl 1995). The sclerotome is formed by the epithelio-mesenchymal transition of the ventral half of the somite and gives rise to the vertebral column, ribs, neural arches, and meninges (Christ et al. 2000, 2004; Christ and Wilting 1992; Verbout 1985). Only the dorsal part of the somite, the dermomyotome, retains its epithelial structure for some time and yields various derivates, such as skeletal muscles (Huang and Christ 2000; Yusuf and Brand-Saberi 2006), smooth muscles (Ben-Yair and Kalcheim 2008), dermis (Ben-Yair et al. 2003; Ben-Yair and Kalcheim 2005; Kalcheim et al. 1999; Mauger 1972a, b; Olivera-Martinez et al. 2000, 2002, 2004), angiogenic cells (Wilting et al. 1995, 2000, 2001), and locally also cartilage (Huang and Christ 2000; reviewed by Yusuf and Brand-Saberi 2012).

The dermomyotome can be divided into two portions in terms of their anatomical localization, the dorsomedial and the ventrolateral portion. The dorsomedial dermomyotome is the source of the intrinsic back muscles, developing from the epaxial myotome, whereas the ventrolateral lip of the dermomyotome (VLL) gives rise to the hypaxial myotome that will form the muscles of the ventrolateral body wall including the limbs (Christ and Ordahl 1995; Huang and Christ 2000). Depending on the somitic level, two different populations of hypaxial muscle precursor cells can be distinguished. At the cranial, cervical and interlimb levels, the dermomyotomes and hypaxial myotomes form proliferating muscle buds to generate the body wall muscles, such as ventral cervical, intercostal, and abdominal muscles (Cinnamon et al. 1999; Ordahl and Le Douarin 1992). In contrast, at the occipital and the fore- and hindlimb levels, premyogenic progenitors delaminate from the ventrolateral dermomyotomes, become committed and undertake a long-range migration to their final destinations: the limbs, the tongue and, in mice, the diaphragm (Chevallier et al. 1977; Christ et al. 1974, 1977; Christ and Ordahl 1995; Huang et al. 1999; Ordahl and Le Douarin 1992; reviewed by Noden and Francis-West 2006). A subpopulation of hypaxial migratory cells, however, use these locations only as a transient repository and subsequently migrate back to the trunk.

3 Secondary Trunk Muscles

At the axial trunk level, two groups of muscles can be distinguished with respect to their medial-lateral distribution. The deeper dorsomedially located trunk muscles, also referred to as epaxial or intrinsic back muscles, constitute the first group. They originate from the dorsomedial dermomyotome located in the immediate vicinity of their future position and are therefore considered as primary trunk muscles (primaxial). The ventrolateral dermomyotome gives rise to segmentally arranged hypaxial muscle only in the thoracic region where they form ventral

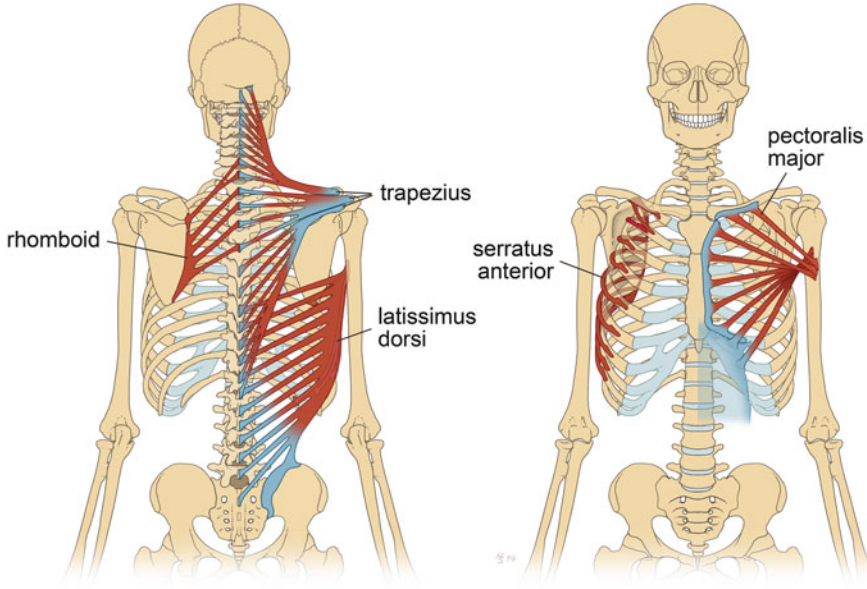


Fig. 1 Schematic representation of human pectoral girdle musculature. Dorsal and ventral views of the human thorax with deep pectoral girdle muscles on the *right* and superficial on the *left* body sides, respectively. The pectoralis minor has been omitted for clarity. The deep pectoral girdle muscles rhomboid and serratus anterior develop directly locally from the somites. By contrast, the superficial pectoral girdle muscles latissimus dorsi dorsally and pectoralis major ventrally develop by the "In–Out" mechanism, comprising the initial migration of precursor cells into the forelimb bud and subsequent retrograde migration towards the main body axis. These muscles can thus be considered as secondary trunk musculature. The trapezius and sternocleidomastoid (not shown) muscle have partially a different developmental source, as it has been shown that its homologue in avians, the cucullaris muscle arises from the occipital lateral plate mesoderm (reviewed by Huang et al. 2014)

dermomyotome/myotome extensions that yield the intercostal muscles. In doing so, they invade the adjacent lateral plate mesoderm in coherent epithelial-like cell groups. In the abdominal body wall, ventrolateral muscle buds fuse to form the oblique and straight abdominal muscles. At the limb and occipital level, cells migrate as individual mesenchymal cells and give up their segmental origin during migration. All migratory hypaxial progenitor cells thus belong to the abaxial domain, because they leave their sites of origin, the paraxial mesoderm, to invade the lateral plate mesoderm. The second group consists of more superficial trunk muscles, which do not arise locally, but undergo a distinct complex procedure of development (Ordahl and Le Douarin 1992; Yusuf and Brand-Saberi 2006, 2012). Recently, the development mechanisms of secondary trunk muscles have been extensively investigated by our and other research groups with regard to the pectoral girdle and cloacal/perineal muscles. In the following, we discuss the results of these studies in order to contribute to a classification of primary and secondary

trunk muscles based on current developmental and molecular evidence, as well as anatomical background information (Fig. 1).

The forelimbs of vertebrates can be anatomically divided into two portions: the part distally to the gleno-humeral joint (stylopod, zeugopod, and autopod) and the proximal pectoral girdle (scapula and clavicle), which connects the forelimb to the trunk (Valasek et al. 2011). All the muscles associated with both the distal and the proximal part, are referred to as brachial muscles. Already decades ago, Beresford et al. demonstrated the common somitic origin of the entire brachial musculature including shoulder muscles such as the deltoid, the pectorales major et minor and the latissimus dorsi, by using quail–chicken chimera studies (Beresford et al. 1978; Beresford 1983).

Whereas much work has been dedicated to the study of the development of the forelimb muscles distal to the gleno-humeral joint, the mechanisms of the formation of proximal pectoral girdle muscles remained, however, poorly understood for a long time. Several groups have suggested that, in contrast to intrinsic back muscles, the superficial pectoral girdle muscles do not develop locally, but originate from the premuscle masses in the forelimb by secondarily migrating from the limb bud to the trunk (Beresford et al. 1978; Grim 1971; Lanser and Fallon 1987; Nagashima et al. 2009). Most recently, by using transplantation techniques in avians, Valasek and colleagues could experimentally confirm that the superficial pectoral girdle muscles pectoralis major and latissimus dorsi arise from myogenic precursor cells which initially migrate from the somites into the forelimb buds and subsequently return to the thorax. This mode of muscle development has been designated as the "In–Out mechanism" and the muscles formed in this manner can be considered as secondary trunk muscles (Evans et al. 2006; Valasek et al. 2011). Furthermore, it is believed that deep muscles of the shoulder girdle, such as the rhomboidei and avian serrati, develop locally from the somites by the same mechanism as the intrinsic body wall muscles, by means of myotomal extensions. In consideration of their in situ hybridization data using MyoD as a myogenic marker in mouse embryos, Valasek et al. assume the same developing mechanisms in mammals (Valasek et al. 2011; Yusuf and Brand-Saberi 2012).

Finally, these different developing modes are reflected by distinct innervations of these two muscle groups. The rhomboidei and the serratus anterior are innervated by direct branches of the ventral spinal nerve roots in the cervical region which belong to the supraclavicular portion of the brachial plexus: N. dorsalis scapulae and N. thoracicus longus, respectively. The innervations of the secondary trunk muscles, pectorales and latissimus dorsi, are in contrast provided via nerves from the fascicles of the brachial plexus (Pars infraclavicularis), namely the medial and lateral pectoral nerves and the thoracodorsal nerve, respectively. Moreover, the paths of these nerves in adult organisms correlate with the migration events during embryonic development of corresponding muscles. Hence, the axons pass from the cervical spinal roots into the brachial plexus towards the axillary region and then project back to the axial trunk (Valasek et al. 2011; Williams and Bannister 1995). Based on these findings, Valasek et al. propose the nomenclature of pectoral girdle musculature as "deep" and "superficial" pectoral girdle muscles.

The "In–Out" mechanism deployed during formation of pectoral girdle musculature has been initially discovered in the context of another group of secondary trunk muscles, perineal muscles in mammals or cloacal muscles in avians. According to the study of Valasek et al. from 2005, the cloacal myogenic precursors in chicken embryos first migrate from the somites into the developing hindlimbs, followed by an extension of a subgroup of progenitor cells from the ventral leg pre-muscle mass towards the cloacal tubercle. Analogous to these findings in birds, they could show that the perineal muscles in mice also develop by initially migrating towards the ventral pre-muscle masses of the hindlimb and subsequently returning back towards the midline. These findings are in line with the observation that the perineal muscles in chicken *limbless* autosomal recessive mutants are completely absent, indicating that the normal development of the hindlimb muscles is a prerequisite for the formation of perineal muscles (Prahlad et al. 1979; Valasek et al. 2005). Considering the molecular mechanisms of this "In–Out" migration, it is interesting to point out that two different modes are used during the two phases. During the initial migration towards the hindlimb, the cloacal myogenic precursors move as single cells expressing Pax3 but not MyoD. However in the course of the retrograde migration, a group of cloacal muscle progenitors extends from the ventral leg towards the cloacal tubercle and expresses Pax3 and MyoD simultaneously during this period. This co-expression of Pax3 and MyoD resembles the myotomal extension during the formation of the body wall muscles and thus represents a type of migration that differs from that during the "In"-phase of the pectoral girdle muscle progenitors (Valasek et al. 2005).

Several malformations of human secondary trunk muscles have been described in the past. Whereas birds retain the cloaca during adult lives, in humans the cloaca only represents an embryological structure, which later develops into rectum, urethra and, in females, vagina. Thus, one of the most severe anomalies of the caudal trunk part is the *persistent cloaca* in humans, which is associated with a very poor prognosis and requires urgent surgical intervention (Hartwig et al. 1991; Inomata et al. 1989; Keith et al. 2005). Furthermore, several cases of innate deficiency of pectoral girdle musculature are presently known (Bergman et al. 1988; David and Winter 1985; Hegde and Shokeir 1982; Paraskevas and Raikos 2010). The most frequent congenital disease of this muscle group is Poland syndrome, characterized by unilateral absence or hypoplasia of pectoralis major and pectoralis minor muscle as well as by ipsilateral skeletal, vascular, and surface feature anomalies (Baban et al. 2009; Jones 1926; Poland 1841). Additionally, cases of asymptomatic anatomical variants of some shoulder girdle muscles have previously been reported. For example, the sternalis muscle is a variation of the anterior superficial body wall musculature, which lies perpendicular to the pectoralis major muscle and parallel to the sternum (Bailey and Tzarnas 1999; Raikos et al. 2011a, b). The existence of all these congenital malformations and anomalies is not surprising, given the fact that the development of the secondary trunk muscles is a very complex process comprising numerous sequential events most of which are regulated by local interactions, which makes it vulnerable for a wide range of defects.

The discovery of this new "In–Out" migration mechanism substantiates the classification in primary and secondary trunk muscles from the developmental point of view and makes further investigations concerning the molecular players involved in this complex process necessary. Insights into the underlying genetic and molecular mechanisms will help us to understand the aetiology of such congenital malformations attaining to the secondary trunk muscles.

4 Head and Neck Musculature

Although, as mentioned earlier, most skeletal muscles throughout the body originate in the somites, the craniofacial muscles go back to several different sources including the paraxial mesoderm. Whereas at other levels, the paraxial mesoderm forms epithelial segments (somites), which undergo further morphological transitions, the head muscles develop from the unsegmented preotic part of the paraxial mesoderm, which retains its mesenchymal structure (Nathan et al. 2008; reviewed by Noden and Francis-West 2006).

Among the craniofacial muscles, four functional groups can be distinguished:

(1) External eye muscles, (originating from the paraxial and prechordal mesoderm), (2) branchiomeric muscles, composed of jaw and facial muscles (derived from the paraxial mesoderm temporarily situated in the branchial arches), (3) tongue and intrinsic laryngeal muscles, derived from the somites. Finally, (4) the neck muscles responsible for the stabilization and free movement of the head originate from the somites (reviewed by Grifone and Kelly 2007; reviewed by Noden and Francis-West 2006; Rios and Marcelle 2009; Sambasivan et al. 2011; reviewed by Yusuf and Brand-Saberi 2012).

In addition to the distinct origin of the head muscles, the molecular control mechanisms during their development differ significantly from those during trunk muscle formation, especially during early developmental stages (Tzahor et al. 2003; Nathan et al. 2008; Mootoosamy and Dietrich 2002; Sambasivan et al. 2009). Whereas trunk muscle precursors express Pax3 before the initiation of the myogenic differentiation program by MyoD, the expression of this transcription factor is missing in head muscle precursors in chicken embryos (Hacker and Guthrie 1998). This is in line with observations in Pax:Myf5 mutant mice, which present a compromised formation of trunk muscles, while head muscles are present (Tajbakhsh et al. 1997). Instead, the developmental program of head muscle precursor cells is regulated by other transcription factors, such as Tbx1, Pitx2, Isl1, Tcf21(capsulin), Msc (MyoR), and Myf5 (Dastjerdi et al. 2007; Dong et al. 2006; Hacker and Guthrie 1998; Mootoosamy and Dietrich 2002; Shih et al. 2007). Furthermore, the head muscle precursors show a converse response to certain molecular signals as compared to the trunk myogenic progenitors. Thus, the Wnt and Shh signalling stimulates trunk myogenesis, whereas head myogenesis is inhibited by these genes (Mootoosamy and Dietrich 2002; Tzahor et al. 2003). In addition to these distinguishing criteria, it should also be mentioned that the

connective tissue of the head musculature takes its origin from a different source than that of the trunk muscle, namely from neural crest cells. On the other hand, the connective tissue of trunk muscles forms locally or from the lateral plate mesoderm (Christ et al. 1974, 1982; Matsuoka et al. 2005; Noden and Francis-West 2006; Noden and Trainor 2005). Most interestingly, head muscle satellite cells share molecular characteristics with heart muscle (reviewed by Tzahor 2014).

Recently, it has been reported that several neck muscles develop from a non-somitic tissue: the lateral plate mesoderm. In their work from 2010, Theis and colleagues show that the cucullaris muscle in chicken, which is a homologue to the human trapezius and sternocleidomastoid muscles, mainly arises from the somatopleure adjacent to somites 1, 2 and 3. Most strikingly, they hereby identify a completely new and hitherto as such unknown myogenic source. Moreover, they demonstrated that the cucullaris muscle and the equivalent muscles in mouse embryos deploy the molecular development program used by head muscles and, in concordance with these observations, their connective tissue originates from the neural crest (Kuratani 2008; Theis et al. 2010; reviewed by Huang et al. 2014).

5 Molecular Mechanisms During the Development of Limb Musculature

The development of (hypaxial) limb musculature is a complex process consisting of five successive phases: establishment of the migrating myogenic precursor pool in the ventrolateral dermomyotome, detachment of the migrating cells from the VLL, migration along defined migratory routes to their target locations, simultaneous proliferation and, finally, their differentiation into muscle fibres. Over the last few decades, a considerable amount of work has focused on the molecular mechanisms controlling these steps of limb muscle development. The following section provides an overview of the most important regulatory factors.

One of these control genes is Pax3, which belongs to the Pax-gene family and encodes a paired domain/homeodomain transcription factor. Pax3 is crucial for the correct establishment of the myogenic progenitor pool in the ventrolateral dermomyotome. Although initially expressed throughout the entire somite, over the course of development, Pax3 becomes restricted to the dermomyotome and is finally upregulated in its ventrolateral lip (VLL). It is noteworthy that Pax3 is expressed at all axial levels of the trunk and thus in both the migrating and non-migrating precursor cells (Goulding et al. 1994; Williams and Ordahl 1994). In the limb muscle progenitor cells, the expression of Pax3 also persists during their long-range migration. Mice deficient in the Pax3 gene, called *Splotch* mice, reveal an impaired myogenic progenitor pool in the VLL, which leads to a lack of limb muscles (Bober et al. 1994; Franz et al. 1993; Tajbakhsh et al. 1997). Another paired box transcription factor involved in limb muscle development is Pax7. Whereas in chicken embryos Pax7 is expressed in migrating myogenic precursors

during the entire migration period, murine muscle progenitors only start to express Pax7 after entering the limb bud mesenchyme (Mansouri et al. 1996; Marcelle et al. 1995).

The tyrosine kinase receptor c-Met and its ligand Scatter Factor/Hepatocyte Growth Factor (SF/HGF) play a critical role in the delamination of migrating myogenic precursors from the VLL. The receptor c-Met is expressed in the ventrolateral and the dorsomedial lip of the dermomyotome, and like Pax3, is expressed by both, the hypaxial and epaxial progenitor cells. Furthermore, its expression extends to all axial levels and thus concerns the migrating as wells as non-migrating myogenic precursors. The migrating myogenic precursors continue to express c-Met during the entire migration period, whereas the ligand SF/HGF is secreted along the migratory pathways and at the final destinations in the limb bud mesenchyme. The role of this signalling system during delamination has been experimentally shown by application of SF/HGF to mesenchyme at intersomitic levels, where the VLL normally does not release any individually migrating cells. In this experimental setup, the application of SF/HGF led to ectopic delamination of myogenic precursor cells (Brand-Saberi et al. 1996a, b; Heymann et al. 1996). In murine c-Met mutants, the migratory hypaxial myogenic progenitors fail to delaminate, resulting in a lack of muscle groups deriving from these precursor cells, namely muscles of the limbs, shoulders, diaphragm, and the hypoglossal cord. Interestingly, other skeletal muscles originating from myogenic precursors retaining their epithelial or myotomal organization, like deep back and intercostal muscles, were not compromised. Thus, the essential function of c-Met and SF/HGF specifically in the development of musculature that derives from individually migrating hypaxial progenitors has been demonstrated (Bladt et al. 1995; Dietrich et al. 1999). In addition to its role in delaminating from the VLL, this ligand–receptor pair also increases the motility of the muscle progenitors and inhibits their differentiation during the migration period (Scaal et al. 1999; reviewed by Birchmeier and Brohmann 2000).

Another transcription factor, Lbx1, enables the directed migration of limb muscles precursors to their target locations in the developing limb buds. While first expressed by the migrating myogenic precursors in the ventrolateral dermomyotome, the expression of this homeobox gene is downregulated as the differentiation program is activated (Mennerich et al. 1998; Dietrich et al. 1998). Notably, Lbx1 is the only gene in the context of hypaxial limb muscle development, which is expressed exclusively by migrating myogenic precursors (Jagla et al. 1995). Although defects in Lbx1 in mice do not compromise the delamination of limb muscle precursors from the VLL, the cells migrate in an erratic manner and thus do not reach their actual destinations, demonstrating the essential role of Lbx1 for navigation of limb muscle precursors along their migratory pathways. Interestingly, in these mutant mice almost all hindlimb muscles as well as the extensor muscles in the forelimbs are missing, whereas the forelimb flexor muscles are only reduced in size. Beside this, it is remarkable that other hypaxial muscle groups requiring precursor cell migration during their development, i.e. the muscles of the diaphragm and the tongue, are not affected in the Lbx1 mutants, pointing towards a

selective function of this gene in the navigation of certain subpopulation of migrating hypaxial muscle precursors (Brohmann et al. 2000; Gross et al. 2000; Schäfer and Braun 1999; reviewed by Yusuf and Brand-Saberi 2012).

Once they arrive at their target locations, the hypaxial myogenic precursor cells begin to express myogenic regulatory factors (MRFs). Until now four MRFs have been identified, namely Myf5, MyoD, MRF4, und Myogenin (Braun et al. 1989, 1990; Edmondson and Olson 1989; Miner and Wold 1990; Rhodes and Konieczny 1989; Rudnicki and Jaenisch 1995; Weintraub et al. 1991). These basic helix-loop-helix transcription factors are responsible for the terminal specification and differentiation of the myogenic progenitors. More precisely, Myf5 and MyoD determine myogenic precursors to myoblasts, whereas Myogenin and MRF4 are critical for myoblast differentiation into muscle fibers (Ott et al. 1991; Pownall and Emerson 1992; Sassoon 1993; reviewed by Brand-Saberi and Christ 1999). Myf5 and MyoD have been assumed to fulfil similar functions in skeletal myogenesis along distinct pathways, as null Myf5 or MyoD mutant mice have muscles, whereas Myf5/MyoD double knockout mice are unable to generate muscle cells (Rudnicki et al. 1993; reviewed by Pownall et al. 2002). It has further been shown that in the absence of Myf5 the activation of the myogenic determination program via MyoD depends on Pax3 (Tajbakhsh et al. 1997).

6 Chemokine Receptor–Ligand Pair CXCR4 and SDF-1

Chemokines are either secreted or membrane-bound small signalling molecules, which act as chemoattractants and are able to induce, through interaction with their receptors, directed cell migration. According to their chemical structure, they are classified in four groups (alpha, beta, gamma and delta) according to the arrangement of the two first cysteine residues that are conserved in all chemokines.

The “stromal-cell-derived factor 1” SDF-1, also designated as CXCL12, is a member of the subfamily of alpha-chemokines, which are characterized by an intervening amino acid separating the two conserved cysteine residues. SDF-1 was first isolated from the murine bone marrow and characterized as pre-B cells growth stimulating factor, giving this chemokine its name (Nagasawa et al. 1994; Shirozu et al. 1995). SDF-1 is highly conserved across species barriers (Burger and Kipps 2006; Shirozu et al. 1995). Its receptor CXCR4 is a 7-transmembrane-domain G-protein coupled receptor, which is widely expressed in different cell types throughout the organism (Nagasawa et al. 1998).

The interaction of SDF-1 with CXCR4 activates a number of different G-protein related signalling pathways and therefore results in a variety of biological responses. Thus, the activated $G\alpha_i$ can inhibit adenylate cyclase as well as activate the Src family of tyrosine kinases, whereas the β and γ subunits activate phospholipase C-β (PLC-ß), phosphoinositide-3-kinase (PI3K) and the Rho pathway (Busillo and Benovic 2007). Furthermore, two potential G-protein-independent pathways following the CXCR4 activation by SDF-1 have been suggested. The

SDF-1 binding induces the tyrosine activation of CXCR4 which leads to the activation of the JAK/STAT pathway (Vila-Coro et al. 1999). Another response to the SDF-1 stimulation of CXCR4 is that of ERK and p38 activation, which itself is partially dependent on arrestin-3 (Cheng et al. 2000; Sun et al. 2002). All these signalling cascades lead to different responses such as cell migration, adhesion or transcriptional activation (Busillo and Benovic 2007; Kucia et al. 2004).

The CXCR4/SDF-1 axis is known to play a key role in numerous developmental, inflammatory, and pathological processes. Thus, it has been shown to be implicated in neurogenesis (Bagri et al. 2002; Lazarini et al. 2003; Pujol et al. 2005), hematopoiesis (Ma et al. 1998; Zou et al. 1998), vascularization (Tachibana et al. 1998), recruitment of T-lymphocyte to sites of immune and inflammatory response (Bleul et al. 1996a, b), and HIV pathogenesis (Deng et al. 1996; Doranz et al. 1996; Feng et al. 1996; Nagasawa et al. 1996).

Although the emphasis in investigations of the CXCR4/SDF-1 axis has initially been laid on hematopoietic stem cells, in the last few years, an extensive body of work from several groups has provided evidence that this ligand–receptor pair is also implicated in the trafficking of stem cells for various tissues during development, tissue injury and regeneration (Kucia et al. 2005, 2006). SDF-1 and CXCR4 have thus been demonstrated to play a crucial role in the migration of several stem cells during embryogenesis, such as migration of primordial germ cells towards the gonads in zebrafish, avians, and mammals (Doitsidou et al. 2002; Knaut et al. 2003; Stebler et al. 2004) or migration of myogenic precursors, which is explicitly discussed below. Moreover, CXCR4 is expressed in murine pluripotent embryonic stem cells and as such, can be considered as a universal stem cell marker (Kucia et al. 2005). Furthermore, Kucia and coworkers postulated that metastasis of cancer cells and trafficking of normal stem cells depend upon very similar mechanisms (Kucia et al. 2005). A widely accepted view is that malignant tumors occur through mutations in normal stem cells (Dontu et al. 2003; Singh et al. 2004). Thus, similarly to normal stem cells, it has been demonstrated that numerous cancer stem cells express CXCR4 on their surface and follow a SDF-1 gradient during their metastasis to other organs. In this way, the CXCR4/SDF-1 axis is essentially involved in the metastasis of numerous cancer types, such as breast, ovarian, and prostate cancer as well as neuroblastoma and rhabdomyosarcoma (Balkwill 2004; Geminder et al. 2001; Hiratsuka et al. 2011; Libura et al. 2002; Müller et al. 2001; Porcile et al. 2004; Sun et al. 2003; reviewed by Kucia et al. 2005).

Interestingly, CXCR4 and SDF-1 are also implicated in muscle regeneration in adult organisms. Skeletal muscles employ self-repair mechanisms after an injury or a structural muscle disease. Hereby, the satellite cells, which are small cells located between the basal lamina and the myofiber membrane, serve as myogenic progenitor cells for growth and repair in mature skeletal muscle tissue (Mauro 1961; Zammit 2008; reviewed by Scharner and Zammit 2011; reviewed by Sambasivan and Tajbakhsh 2014). Recently, it has been shown that the mobilization of CXCR4 expressing satellite cells depends on SDF-1 signalling and therefore the CXCR4/SDF-1 axis plays a pivotal role in skeletal muscle regeneration (Brzoska et al. 2012; Ratajczak et al. 2003).

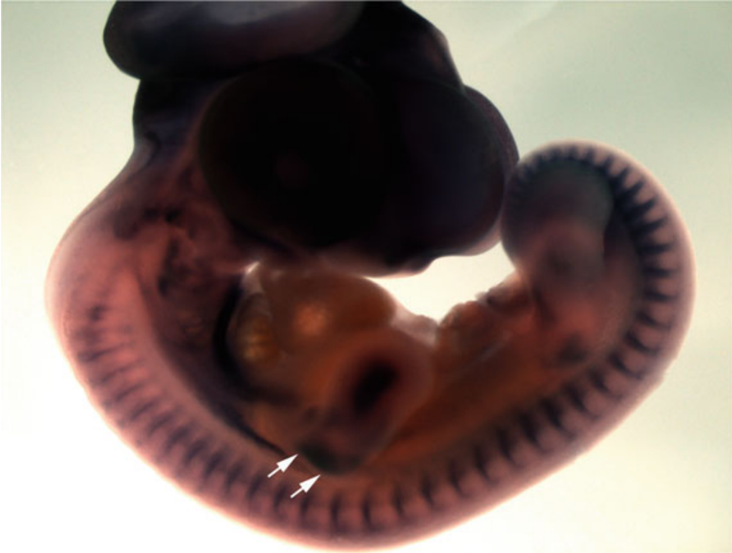


Fig. 2 In situ hybridization analysis of SDF-1 in HH26 chicken embryo. At this developmental stage, the expression of SDF-1 is not only detectable in the forelimb mesenchyme but also in the shoulder region (*white arrows*), where the later pectoral girdle musculature develops. Thus, SDF-1 serves as a guiding cue for the CXCR4-expressing pectoral girdle muscle precursors during their retrograde migration

7 The Role of CXCR4 and SDF-1 During Migration of Myogenic Precursors

The decisive role of the chemokine receptor CXCR4 and its ligand SDF-1 in various migration events during development suggests that they could also be involved in migration of hypaxial myogenic precursors. As described above, two different long-term migration processes during muscle development can be distinguished, namely, first, the anterograde migration of myogenic progenitors from the somites to the limb buds, diaphragm, and tongue *anlage*. The second one is the retrograde migration of a subpopulation of these precursor cells from the limbs towards the trunk to form the cloacal and the pectoral girdle muscles. In the following, we discuss the role of CXCR4/SDF-1 axis during these myogenic migration events.

The assumption above has been strengthened by analyses of the CXCR4 and SDF-1 expression patterns in chicken and mouse embryos carried out by our and other groups (Fig. 2). Similarly to c-met and its ligand SF/HGF, CXCR4 is expressed in migrating myogenic precursors of the limb buds, and at later stages, in typical premuscle domains in the fore- and hindlimbs, while SDF-1 is expressed

by the limb mesenchyme and along the migratory routes (Rehimi et al. 2008; Vasyutina et al. 2005; Yusuf et al. 2005).

First, our and other laboratories have demonstrated that CXCR4 and SDF-1 are required for the migration of hypaxial myogenic precursors in the developing limb buds in avians and mammals (Vasyutina et al. 2005; Odemis et al. 2005; Yusuf et al. 2006). Hereby, the SDF-1 signalling serves as a guidance cue for the $CXCR4^+$ myogenic precursor cells during their migration. Vasyutina et al. demonstrated that ectopic application of SDF-1 in the chicken limb mesenchyme leads to an attraction of myogenic precursor cells and, hence, affects their normal migration pattern. Furthermore, in $CXCR4^{-/-}$ mutant mouse embryos, they observed a slight increase in the apoptosis rate of myogenic precursor cells in the proximal forelimb, whereas the reduction in numbers of progenitor cells was most pronounced in the distal limb (Vasyutina et al. 2005). Strikingly, this decreased number of myogenic precursors is temporarily compensated for by other mechanisms, allowing continued development, but nevertheless resulting in a reduction of limb muscle mass in late embryonic stages (Odemis et al. 2005). By contrast, in Gab1-CXCR4 double-mutant mouse embryos, the forelimb muscles were more severely reduced in size, pointing to a convergence of CXCR4 signalling with the signal transduction effectors used by Gab1 (Vasyutina et al. 2005). Work from our laboratory has shown the importance of the CXCR4/SDF-1 axis for the migration of forelimb muscle precursors in chicken embryos by manipulating the CXCR4 signalling with CXCR4-inhibitors T140 and TN14003. Application of these inhibitors in the forelimb mesenchyme affected the migration of $CXCR4^+$ myogenic precursors from the VLL into the limb bud (Yusuf et al. 2006). It is noteworthy, that the $CXCR4^+$ migrating forelimb muscle precursor cells only represent a subpopulation of the entire $Pax3^+$ cell pool. Therefore, inhibition of the CXCR4 signalling only causes a decrease of limb muscles masses and never a complete absence. Moreover, it has been shown that some of the $CXCR4^+$ cells are both myogenic and endothelial precursors, making the designation “premyogenic precursor cells” more accurate (Vasyutina et al. 2005; Yusuf et al. 2005, 2006; Yusuf and Brand-Saberi 2006, 2012).

In 2010, our group demonstrated the role of the CXCR4/SDF-1 axis in the retrograde migration during the development of cloacal muscles in chicken embryos. A pronounced expression domain of SDF-1 was detected in the cloacal region and CXCR4 expression was present in the ventral hindlimb and around the cloaca. Subsequently to the detailed analysis of the SDF-1 and CXCR4 expression pattern in chicken embryos, experimental manipulations of the CXCR4/SDF-1 signalling were carried out during the period of the retrograde migration. Similar to the defects in migration of forelimb muscle precursors, insertion of CXCR4-inhibitors or SDF-1 expressing cells into the ventral proximal hindlimb mesenchyme affected the migration of myogenic precursor cells from the hindlimbs towards the cloacal tubercle and resulted in a reduction of cloacal musculature in later stages (Rehimi et al. 2010). Hereby, the CXCR4-inhibitors in the path of $CXCR4^+$ myogenic precursors hindered them in their migration, whereas the SDF-1 expressing cells attracted the CXCR4 expressing progenitors, which led to their accumulation around the SDF-1 source and, finally, also affected their directed

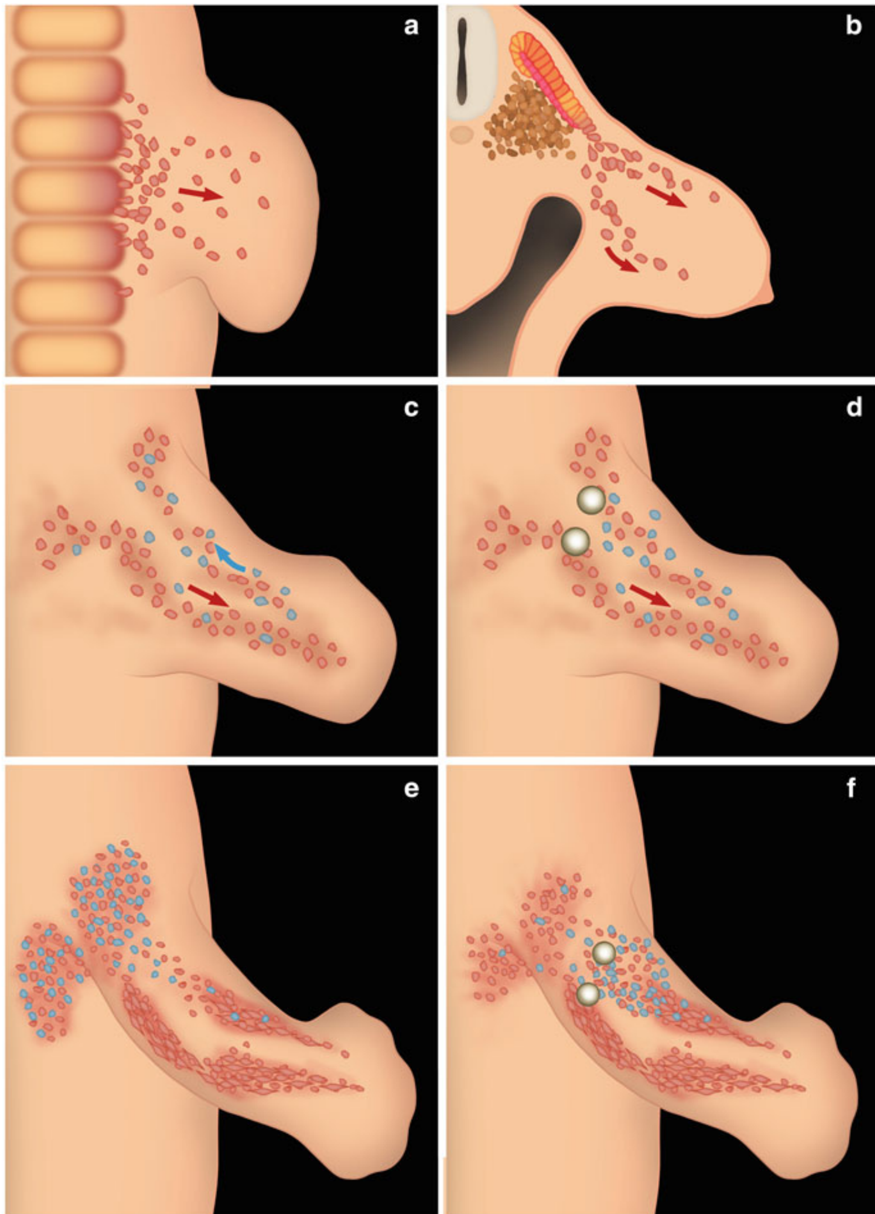


Fig. 3 Schematic representation of the regular and experimentally inhibited development of the superficial pectoral girdle muscles (**a** and **b**). The myogenic precursor cells delaminate from the ventrolateral dermomyotomal lip of the somites and migrate dorsally and ventrally into the developing forelimb bud (**c**). At a later developmental stage several myogenic precursors undergo a retrograde migration from the forelimb bud towards the trunk, (**e**) where they finally proliferate and differentiate into muscle cells to form the superficial pectoral girdle musculature. This developing mode as referred to as "In–Out" mechanism and the muscles formed in this manner can be considered as secondary trunk muscles (**d**). In the experimental setup, acrylic beads soaked with a CXCR4 inhibitor solution hinder the myogenic precursors in their retrograde migration, (**f**)

retrograde migration. Thus, using the example of cloacal musculature, it has been shown that CXCR4 and SDF-1 also play an essential role for the "Out"- phase of the "In–Out" mechanism during the development of secondary trunk muscles.

More recently, we have performed lineage tracing experiments in a similar way at the level of the pectoral girdle, employing electroporation of TOL2-EGFP and then monitoring the presence of EGFP-positive cells in different phases of their migration into the limb bud and back to the trunk. Additionally, we could demonstrate by live imaging on transversally sectioned tissue blocks of electroporated embryos that SDF-1 signalling is required for retrograde migration to occur (Masyuk et al. 2014). The CXCR4-inhibitors T140 and TN14003 were adsorbed to carrier beads and grafted into the proximal right forelimb bud of chicken embryos of HH-stage 23. We could show that cells migrate as individual mesenchymal cells that can only penetrate towards their proximal target at the shoulder girdle, if SDF-1-CXCR4 signalling is undisturbed (Fig.3).

To conclude, in recent years a wide range of investigations have been carried out to reveal the importance of the chemokine receptor CXCR4 and its ligand SDF-1 for the migration of myogenic precursors during development of hypaxial musculature. There is evidence that muscle precursors for the secondary sites of myogenesis, such as the cloaca and the shoulder girdle, are recruited from the pool of Pax7-positive myogenic precursors, at least during development. Further research is necessary to clarify the cellular processes in the context of a possible role of CXCR4 and SDF-1 for other migration processes during adult myogenesis and embryological development in general.

Acknowledgements The authors are grateful for the excellent technical support of Swantje Wulff, Rana Houmany, and Anke Lodwig. We furthermore thank our co-authors in original papers quoted, in particular, Carsten Theiss, Faisal Yusuf, and Gabriela Morosan-Puopolo for their excellent work. The authors' special thanks are due to Helga Schulze for preparing the artwork of this article. We thank Daniel Terheyden-Keighley for his advice regarding language aspects of this manuscript. Part of the original work reviewed here has been supported by the DFG (Br 957/5-1, 5-2, 5-3), and the EU's Sixth Framework Network of Excellence MYORES (511978).

References

Askari AT, Unzek S, Popovic ZB, Goldman CK, Forudi F, Kiedrowski M, Rovner A, Ellis SG, Thomas JD, DiCorleto PE, Topol EJ, Penn MS (2003) Effect of stromal-cell-derived factor 1 on stem-cell homing and tissue regeneration in ischaemic cardiomyopathy. Lancet 362:697–703

Aulehla A, Pourquié O (2006) On periodicity and directionality of somitogenesis. Anat Embryol 211(Suppl 1):3–8

Fig. 3 (continued) which leads to a disturbed development and reduction of the superficial shoulder musculature. Thus, the CXCR4/SDF-1 axis is involved in the retrograde migration required for the pectoral girdle muscle development

Baban A, Torre M, Bianca S, Buluggiu A, Rossello MI, Calevo MG, Valle M, Ravazzolo R, Jasonni V, Lerone M (2009) Poland syndrome with bilateral features: case description with review of the literature. Am J Med Genet A 149A(7):1597–1602

Bagri A, Gurney T, He X, Zou Y, Littman DR, Tessier-Lavigne M, Pleasure SJ (2002) The chemokine SDF1 regulates migration of dentate granule cells. Development 129(18):4249–4260

Bailey PM, Tzarnas CD (1999) The sternalis muscle: a normal finding encountered during breast surgery. Plast Reconstr Surg 103(4):1189–1190

Balkwill F (2004) The significance of cancer cell expression of the chemokine receptor CXCR4. Semin Cancer Biol 14(3):171–179

Ben-Yair R, Kalcheim C (2005) Lineage analysis of the avian dermomyotome sheet reveals the existence of single cells with both dermal and muscle progenitor fates. Development 132 (4):689–701

Ben-Yair R, Kalcheim C (2008) Notch and bone morphogenetic protein differentially act on dermomyotome cells to generate endothelium, smooth, and striated muscle. J Cell Biol 180 (3):607–618

Ben-Yair R, Kahane N, Kalcheim C (2003) Coherent development of dermomyotome and dermis from the entire mediolateral extent of the dorsal somite. Development 130(18):4325–4336

Beresford B (1983) Brachial muscles in the chick embryo: the fate of individual somites. J Embryol Exp Morphol 77:99–116

Beresford B, Le Lievre C, Rathbone MP (1978) Chimaera studies of the origin and formation of the pectoral musculature of the avian embryo. J Exp Zool 205(2):321–326

Bergman RA, Thompson SA, Saadeh FA (1988) Anomalous fascicle and high origin of latissimus dorsi compensating for absence of serratus anterior. Anat Anz 167(2):161–164

Birchmeier C, Brohmann H (2000) Genes that control the development of migrating muscle precursor cells. Curr Opin Cell Biol 12(6):725–730

Bladt F, Riethmacher D, Isenmann S, Aguzzi A, Birchmeier C (1995) Essential role for the c-met receptor in the migration of myogenic precursor cells into the limb bud. Nature 376 (6543):768–771

Bleul CC, Farzan M, Choe H, Parolin C, Clark-Lewis I, Sodroski J, Springer TA (1996a) The lymphocyte chemoattractant SDF-1 is a ligand for LESTR/fusin and blocks HIV-1 entry. Nature 382(6594):829–833

Bleul CC, Fuhlbrigge RC, Casasnovas JM, Aiuti A, Springer TA (1996b) A highly efficacious lymphocyte chemoattractant, stromal cell-derived factor 1 (SDF-1). J Exp Med 184(3):1101–1109

Bober E, Franz T, Arnold HH, Gruss P, Tremblay P (1994) Pax-3 is required for the development of limb muscles: a possible role for the migration of dermomyotomal muscle progenitor cells. Development 120(3):603–612

Brand-Saberi B, Christ B (1999) Genetic and epigenetic control of muscle development in vertebrates. Cell Tissue Res 296(1):199–212

Brand-Saberi B, Müller TS, Wilting J, Christ B, Birchmeier C (1996a) Scatter factor/hepatocyte growth factor (SF/HGF) induces emigration of myogenic cells at interlimb level in vivo. Dev Biol 179(1):303–308

Brand-Saberi B, Wilting J, Ebensperger C, Christ B (1996b) The formation of somite compartments in the avian embryo. Int J Dev Biol 40(1):411–420

Braun T, Buschhausen-Denker G, Bober E, Tannich E, Arnold HH (1989) A novel human muscle factor related to but distinct from MyoD1 induces myogenic conversion in 10 T1/2 fibroblasts. EMBO J 8(3):701–709

Braun T, Bober E, Winter B, Rosenthal N, Arnold HH (1990) Myf-6, a new member of the human gene family of myogenic determination factors: evidence for a gene cluster on chromosome 12. EMBO J 9(3):821–831

Brohmann H, Jagla K, Birchmeier C (2000) The role of Lbx1 in migration of muscle precursor cells. Development 127(2):437–445

Brzoska E, Kowalewska M, Markowska-Zagrajek A, Kowalski K, Archacka K, Zimowska M, Grabowska I, Czerwińska AM, Czarnecka-Góra M, Stremińska W, Jańczyk-Ilach K, Ciemerych MA (2012) Sdf-1 (CXCL12) improves skeletal muscle regeneration via the mobilisation of Cxcr4 and CD34 expressing cells. Biol Cell 104(12):722–737

Burger JA, Kipps TJ (2006) CXCR4: a key receptor in the crosstalk between tumor cells and their microenvironment. Blood 107(5):1761–1767

Busillo JM, Benovic JL (2007) Regulation of CXCR4 signaling. Biochim Biophys Acta 1768 (4):952–963

Cheng ZJ, Zhao J, Sun Y, Hu W, Wu YL, Cen B, Wu GX, Pei G (2000) Beta-arrestin differentially regulates the chemokine receptor CXCR4-mediated signaling and receptor internalization, and this implicates multiple interaction sites between beta-arrestin and CXCR4. J Biol Chem 275 (4):2479–2485

Chevallier A, Kieny M, Mauger A (1977) Limb-somite relationship: origin of the limb musculature. J Embryol Exp Morphol 41:245–258

Christ B, Ordahl CP (1995) Early stages of chick somite development. Anat Embryol 191(5):381–396

Christ B, Wilting J (1992) From somites to vertebral column. Ann Anat 174(1):23–32

Christ B, Jacob HJ, Jacob M (1972) Experimentelle Untersuchungen zur Somitenentstehung beim Hühnerembryo (Experimental analysis of somitogenesis in the chick embryo). Z Anat Entwicklungsgesch 138(1):82–97

Christ B, Jacob HJ, Jacob M (1974) Uber den Ursprung der Flügelmuskulatur. Experimentelle Untersuchungen mit Wachtel- und Hühnerembryonen (Origin of wing musculature. Experimental studies on quail and chick embryos). Experientia 30(12):1446–1449

Christ B, Jacob HJ, Jacob M (1977) Experimental analysis of the origin of the wing musculature in avian embryos. Anat Embryol 150(2):171–186

Christ B, Jacob HJ, Jacob M, Wachtler F (1982) On the origin, distribution and determination of avian limb mesenchymal cells. Prog Clin Biol Res 110:281–291

Christ B, Huang R, Wilting J (2000) The development of the avian vertebral column. Anat Embryol 202(3):179–194

Christ B, Huang R, Scaal M (2004) Formation and differentiation of the avian sclerotome. Anat Embryol 208(5):333–350

Cinnamon Y, Kahane N, Kalcheim C (1999) Characterization of the early development of specific hypaxial muscles from the ventrolateral myotome. Development 126(19):4305–4315

Dastjerdi A, Robson L, Walker R, Hadley J, Zhang Z, Rodriguez-Niedenführ M, Ataliotis P, Baldini A, Scambler P, Francis-West P (2007) Tbx1 regulation of myogenic differentiation in the limb and cranial mesoderm. Dev Dyn 236(2):353–363

David TJ, Winter RM (1985) Familial absence of the pectoralis major, serratus anterior, and latissimus dorsi muscles. J Med Genet 22(5):390–392

Deng H, Liu R, Ellmeier W, Choe S, Unutmaz D, Burkhart M, Di Marzio P, Marmon S, Sutton RE, Hill CM, Davis CB, Peiper SC, Schall TJ, Littman DR, Landau NR (1996) Identification of a major co-receptor for primary isolates of HIV-1. Nature 381(6584):661–666

Dietrich S, Schubert FR, Healy C, Sharpe PT, Lumsden A (1998) Specification of the hypaxial musculature. Development 125(12):2235–2249

Dietrich S, Abou-Rebyeh F, Brohmann H, Bladt F, Sonnenberg-Riethmacher E, Yamaai T, Lumsden A, Brand-Saberi B, Birchmeier C (1999) The role of SF/HGF and c-Met in the development of skeletal muscle. Development 126(8):1621–1629

Doitsidou M, Reichman-Fried M, Stebler J, Köprunner M, Dörries J, Meyer D, Esguerra CV, Leung T, Raz E (2002) Guidance of primordial germ cell migration by the chemokine SDF-1. Cell 111(5):647–659

Dong F, Sun X, Liu W, Ai D, Klysik E, Lu M, Hadley J, Antoni L, Chen L, Baldini A, Francis-West P, Martin JF (2006) Pitx2 promotes development of splanchnic mesoderm-derived branchiomeric muscle. Development 133(24):4891–4899

Dontu G, Al-Hajj M, Abdallah WM, Clarke MF, Wicha MS (2003) Stem cells in normal breast development and breast cancer. Cell Prolif 36(Suppl 1):59–72
Doranz BJ, Rucker J, Yi Y, Smyth RJ, Samson M, Peiper SC, Parmentier M, Collman RG, Doms RW (1996) A dual-tropic primary HIV-1 isolate that uses fusin and the beta-chemokine receptors CKR-5, CKR-3, and CKR-2b as fusion cofactors. Cell 85(7):1149–1158
Edmondson DG, Olson EN (1989) A gene with homology to the myc similarity region of MyoD1 is expressed during myogenesis and is sufficient to activate the muscle differentiation program. Genes Dev 3(5):628–640
Evans DJR, Valasek P, Schmidt C, Patel K (2006) Skeletal muscle translocation in vertebrates. Anat Embryol 211(Suppl 1):43–50
Feng Y, Broder CC, Kennedy PE, Berger EA (1996) HIV-1 entry cofactor: functional cDNA cloning of a seven-transmembrane, G protein-coupled receptor. Science 272(5263):872–877
Franz T, Kothary R, Surani MA, Halata Z, Grim M (1993) The Splotch mutation interferes with muscle development in the limbs. Anat Embryol 187(2):153–160
Geminder H, Sagi-Assif O, Goldberg L, Meshel T, Rechavi G, Witz IP, Ben-Baruch A (2001) A possible role for CXCR4 and its ligand, the CXC chemokine stromal cell-derived factor-1, in the development of bone marrow metastases in neuroblastoma. J Immunol 167(8):4747–4757
Goulding M, Lumsden A, Paquette AJ (1994) Regulation of Pax-3 expression in the dermomyotome and its role in muscle development. Development 120(4):957–971
Grifone R, Kelly RG (2007) Heartening news for head muscle development. Trends Genet 23 (8):365–369
Grim M (1971) Development of the primordia of the latissimus dorsi muscle of the chicken. Folia Morphol (Praha) 19(3):252–258
Gross MK, Moran-Rivard L, Velasquez T, Nakatsu MN, Jagla K, Goulding M (2000) Lbx1 is required for muscle precursor migration along a lateral pathway into the limb. Development 127(2):413–424
Hacker A, Guthrie S (1998) A distinct developmental programme for the cranial paraxial mesoderm in the chick embryo. Development 125(17):3461–3472
Hartwig NG, Steffelaar JW, Van de Kaa C, Schueler JA, Vermeij-Keers C (1991) Abdominal wall defect associated with persistent cloaca. The embryologic clues in autopsy. Am J Clin Pathol 96(5):640–647
Hegde HR, Shokeir MH (1982) Posterior shoulder girdle abnormalities with absence of pectoralis major muscle. Am J Med Genet 13(3):285–293
Heymann S, Koudrova M, Arnold H, Köster M, Braun T (1996) Regulation and function of SF/HGF during migration of limb muscle precursor cells in chicken. Dev Biol 180(2):566–578
Hiratsuka S, Duda DG, Huang Y, Goel S, Sugiyama T, Nagasawa T, Fukumura D, Jain RK (2011) C-X-C receptor type 4 promotes metastasis by activating p38 mitogen-activated protein kinase in myeloid differentiation antigen (Gr-1)-positive cells. Proc Natl Acad Sci U S A 108(1):302–307
Huang R, Christ B (2000) Origin of the epaxial and hypaxial myotome in avian embryos. Anat Embryol 202(5):369–374
Huang R, Zhi Q, Izpisua-Belmonte JC, Christ B, Patel K (1999) Origin and development of the avian tongue muscles. Anat Embryol 200(2):137–152
Huang R, Pu Q, Patel K (2014) The lateral plate mesoderm: a novel source of skeletal muscle. In: Brand-Saberi B (ed) Vertebrate myogenesis: stem cells and precursors, Results and problems in cell differentiation. Springer, Heidelberg
Inomata Y, Tanaka K, Ozawa K (1989) Sacral anomaly and pelvic floor muscle in imperforate anus: a clinical and experimental study. Nihon Geka Hokan 58(2):217–230
Jagla K, Dollé P, Mattei MG, Jagla T, Schuhbaur B, Dretzen G, Bellard F, Bellard M (1995) Mouse Lbx1 and human LBX1 define a novel mammalian homeobox gene family related to the Drosophila lady bird genes. Mech Dev 53(3):345–356
Jones HW (1926) Congenital absence of the pectoral muscles. Br Med J 2(3418):59–60

Kalcheim C, Cinnamon Y, Kahane N (1999) Myotome formation: a multistage process. Cell Tissue Res 296(1):161–173

Keith A, George H, Patrick MJ (2005) Pediaric surgery, 4th edn. Elsevier Saunders, Philadelphia, pp 496–517

Klein RS, Rubin JB, Gibson HD, DeHaan EN, Alvarez-Hernandez X, Segal RA, Luster AD (2001) SDF-1 alpha induces chemotaxis and enhances sonic hedgehog-induced proliferation of cerebellar granule cells. Development 128:1971–1981

Knaut H, Werz C, Geisler R, Nüsslein-Volhard C (2003) A zebrafish homologue of the chemokine receptor Cxcr4 is a germ-cell guidance receptor. Nature 421(6920):279–282

Kucia M, Jankowski K, Reca R, Wysoczynski M, Bandura L, Allendorf DJ, Zhang J, Ratajczak J, Ratajczak MZ (2004) CXCR4-SDF-1 signalling, locomotion, chemotaxis and adhesion. J Mol Histol 35(3):233–245

Kucia M, Reca R, Miekus K, Wanzeck J, Wojakowski W, Janowska-Wieczorek A, Ratajczak J, Ratajczak MZ (2005) Trafficking of normal stem cells and metastasis of cancer stem cells involve similar mechanisms: pivotal role of the SDF-1-CXCR4 axis. Stem Cells 23(7):879–894

Kucia M, Reca R, Campbell FR, Zuba-Surma E, Majka M, Ratajczak J, Ratajczak MZ (2006) A population of very small embryonic-like (VSEL) CXCR4(+)SSEA-1(+)Oct-4+ stem cells identified in adult bone marrow. Leukemia 20(5):857–869

Kuratani S (2008) Evolutionary developmental studies of cyclostomes and the origin of the vertebrate neck. Dev Growth Differ 50(Suppl 1):S189–94

Lanser ME, Fallon JF (1987) Development of wing-bud-derived muscles in normal and wingless chick embryos: a computer-assisted three-dimensional reconstruction study of muscle pattern formation in the absence of skeletal elements. Anat Rec 217(1):61–78

Lazarini F, Tham TN, Casanova P, Arenzana-Seisdedos F, Dubois-Dalcq M (2003) Role of the alpha-chemokine stromal cell-derived factor (SDF-1) in the developing and mature central nervous system. Glia 42(2):139–148

Libura J, Drukala J, Majka M, Tomescu O, Navenot JM, Kucia M, Marquez L, Peiper SC, Barr FG, Janowska-Wieczorek A, Ratajczak MZ (2002) CXCR4-SDF-1 signaling is active in rhabdomyosarcoma cells and regulates locomotion, chemotaxis, and adhesion. Blood 100(7):2597–2606

Lu M, Grove EA, Miller RJ (2002) Abnormal development of the hippocampal dentate gyrus in mice lacking the CXCR4 chemokine receptor. Proc Natl Acad Sci USA 99:7090–7095

Ma Q, Jones D, Borghesani PR, Segal RA, Nagasawa T, Kishimoto T, Bronson RT, Springer TA (1998) Impaired B-lymphopoiesis, myelopoiesis, and derailed cerebellar neuron migration in CXCR4- and SDF-1-deficient mice. Proc Natl Acad Sci U S A 95(16):9448–9453

Mansouri A, Hallonet M, Gruss P (1996) Pax genes and their roles in cell differentiation and development. Curr Opin Cell Biol 8(6):851–857

Marcelle C, Wolf J, Bronner-Fraser M (1995) The in vivo expression of the FGF receptor FREK mRNA in avian myoblasts suggests a role in muscle growth and differentiation. Dev Biol 172 (1):100–114

Masyuk M, Abduelmula A, Morosan-Puopolo G, Ödemis V, Rehimi R, Khalida N, Yusuf F, Engele J, Tamamura H, Theiss C, Brand-Saberi B (2014) Retrograde migration of pectoral girdle muscle precursors depends on CXCR4/SDF-1 signaling. Histochem Cell Biol. doi:10.1007/s00418-014-1237-7

Matsuoka T, Ahlberg PE, Kessaris N, Iannarelli P, Dennehy U, Richardson WD, McMahon AP, Koentges G (2005) Neural crest origins of the neck and shoulder. Nature 436(7049):347–355

Mauger A (1972a) Rôle du mésoderme somitique dans le développement du plumage dorsal chez l'embryon de Poulet. I. Origine, capacités de régulation et détermination du mésoderme plumigène (The role of somitic mesoderm in the development of dorsal plumage in chick embryos. I. Origin, regulative capacity and determination of the plumage-forming mesoderm). J Embryol Exp Morphol 28(2):313–341

Mauger A (1972b) Rôle du mésoderme somitique dans le développement du plumage dorsal chez l'embryon de Poulet. II. Régionalisation du mésoderme plumigène (The role of somitic mesoderm in the development of dorsal plumage in chick embryos. II. Regionalization of the plumage-forming mesoderm). J Embryol Exp Morphol 28(2):343–366

Mauro A (1961) Satellite cell of skeletal muscle fibers. J Biophys Biochem Cytol 9:493–495

Mennerich D, Schäfer K, Braun T (1998) Pax-3 is necessary but not sufficient for lbx1 expression in myogenic precursor cells of the limb. Mech Dev 73(2):147–158

Miner JH, Wold B (1990) Herculin, a fourth member of the MyoD family of myogenic regulatory genes. Proc Natl Acad Sci U S A 87(3):1089–1093

Mootoosamy RC, Dietrich S (2002) Distinct regulatory cascades for head and trunk myogenesis. Development 129(3):573–583

Müller A, Homey B, Soto H, Ge N, Catron D, Buchanan ME, McClanahan T, Murphy E, Yuan W, Wagner SN, Barrera JL, Mohar A, Verástegui E, Zlotnik A (2001) Involvement of chemokine receptors in breast cancer metastasis. Nature 410(6824):50–56

Nagasawa T, Kikutani H, Kishimoto T (1994) Molecular cloning and structure of a pre-B-cell growth-stimulating factor. Proc Natl Acad Sci U S A 91(6):2305–2309

Nagasawa T, Nakajima T, Tachibana K, Iizasa H, Bleul CC, Yoshie O, Matsushima K, Yoshida N, Springer TA, Kishimoto T (1996) Molecular cloning and characterization of a murine pre-B-cell growth-stimulating factor/stromal cell-derived factor 1 receptor, a murine homolog of the human immunodeficiency virus 1 entry coreceptor fusin. Proc Natl Acad Sci U S A 93 (25):14726–14729

Nagasawa T, Tachibana K, Kishimoto T (1998) A novel CXC chemokine PBSF/SDF-1 and its receptor CXCR4: their functions in development, hematopoiesis and HIV infection. Semin Immunol 10(3):179–185

Nagashima H, Sugahara F, Takechi M, Ericsson R, Kawashima-Ohya Y, Narita Y, Kuratani S (2009) Evolution of the turtle body plan by the folding and creation of new muscle connections. Science 325(5937):193–196

Nathan E, Monovich A, Tirosh-Finkel L, Harrelson Z, Rousso T, Rinon A, Harel I, Evans SM, Tzahor E (2008) The contribution of Islet1-expressing splanchnic mesoderm cells to distinct branchiomeric muscles reveals significant heterogeneity in head muscle development. Development 135(4):647–657

Noden DM, Francis-West P (2006) The differentiation and morphogenesis of craniofacial muscles. Dev Dyn 235(5):1194–1218

Noden DM, Trainor PA (2005) Relations and interactions between cranial mesoderm and neural crest populations. J Anat 207(5):575–601

Odemis V, Lamp E, Pezeshki G, Moepps B, Schilling K, Gierschik P, Littman DR, Engele J (2005) Mice deficient in the chemokine receptor CXCR4 exhibit impaired limb innervation and myogenesis. Mol Cell Neurosci 30(4):494–505

Olivera-Martinez I, Coltey M, Dhouailly D, Pourquié O (2000) Mediolateral somitic origin of ribs and dermis determined by quail-chick chimeras. Development 127(21):4611–4617

Olivera-Martinez I, Missier S, Fraboulet S, Thélu J, Dhouailly D (2002) Differential regulation of the chick dorsal thoracic dermal progenitors from the medial dermomyotome. Development 129(20):4763–4772

Olivera-Martinez I, Thélu J, Dhouailly D (2004) Molecular mechanisms controlling dorsal dermis generation from the somitic dermomyotome. Int J Dev Biol 48(2–3):93–101

Ordahl CP, Le Douarin NM (1992) Two myogenic lineages within the developing somite. Development 114(2):339–353

Ott MO, Bober E, Lyons G, Arnold H, Buckingham M (1991) Early expression of the myogenic regulatory gene, myf-5, in precursor cells of skeletal muscle in the mouse embryo. Development 111(4):1097–1107

Paraskevas GK, Raikos A (2010) Bilateral pectoral musculature malformations with concomitant vascular anomaly. Folia Morphol (Warsz) 69(3):187–191

Poland A (1841) Deficiency of the pectoral muscles. Guys Hosp Rep 6:191–193

Porcile C, Bajetto A, Barbero S, Pirani P, Schettini G (2004) CXCR4 activation induces epidermal growth factor receptor transactivation in an ovarian cancer cell line. Ann N Y Acad Sci 1030:162–169

Pownall ME, Emerson CP (1992) Sequential activation of three myogenic regulatory genes during somite morphogenesis in quail embryos. Dev Biol 151(1):67–79

Pownall ME, Gustafsson MK, Emerson CP (2002) Myogenic regulatory factors and the specification of muscle progenitors in vertebrate embryos. Annu Rev Cell Dev Biol 18:747–783

Prahlad KV, Skala G, Jones DG, Briles WE (1979) Limbless: a new genetic mutant in the chick. J Exp Zool 209(3):427–434

Pujol F, Kitabgi P, Boudin H (2005) The chemokine SDF-1 differentially regulates axonal elongation and branching in hippocampal neurons. J Cell Sci 118(Pt 5):1071–1080

Raikos A, Paraskevas GK, Tzika M, Faustmann P, Triaridis S, Kordali P, Kitsoulis P, Brand-Saberi B (2011a) Sternalis muscle: an underestimated anterior chest wall anatomical variant. J Cardiothorac Surg 6:73

Raikos A, Paraskevas GK, Yusuf F, Kordali P, Ioannidis O, Brand-Saberi B (2011b) Sternalis muscle: a new crossed subtype, classification, and surgical applications. Ann Plast Surg 67 (6):646–648

Ratajczak MZ, Majka M, Kucia M, Drukala J, Pietrzkowski Z, Peiper S, Janowska-Wieczorek A (2003) Expression of functional CXCR4 by muscle satellite cells and secretion of SDF-1 by muscle-derived fibroblasts is associated with the presence of both muscle progenitors in bone marrow and hematopoietic stem/progenitor cells in muscles. Stem Cells 21(3):363–371

Rehimi R, Khalida N, Yusuf F, Dai F, Morosan-Puopolo G, Brand-Saberi B (2008) Stromal-derived factor-1 (SDF-1) expression during early chick development. Int J Dev Biol 52(1):87–92

Rehimi R, Khalida N, Yusuf F, Morosan-Puopolo G, Brand-Saberi B (2010) A novel role of CXCR4 and SDF-1 during migration of cloacal muscle precursors. Dev Dyn 239(6):1622–1631

Rhodes SJ, Konieczny SF (1989) Identification of MRF4: a new member of the muscle regulatory factor gene family. Genes Dev 3(12B):2050–2061

Rios AC, Marcelle C (2009) Head muscles: aliens who came in from the cold? Dev. Cell 16 (6):779–780

Rudnicki MA, Jaenisch R (1995) The MyoD family of transcription factors and skeletal myogenesis. Bioessays 17(3):203–209

Rudnicki MA, Schnegelsberg PN, Stead RH, Braun T, Arnold HH, Jaenisch R (1993) MyoD or Myf-5 is required for the formation of skeletal muscle. Cell 75(7):1351–1359

Sambasivan R, Tajbakhsh S (2014) Adult skeletal muscle stem cells. In: Brand-Saberi B (ed) Vertebrate myogenesis: stem cells and precursors, Results and problems in cell differentiation. Springer, Heidelberg

Sambasivan R, Gayraud-Morel B, Dumas G, Cimper C, Paisant S, Kelly RG, Kelly R, Tajbakhsh S (2009) Distinct regulatory cascades govern extraocular and pharyngeal arch muscle progenitor cell fates. Dev Cell 16(6):810–821

Sambasivan R, Kuratani S, Tajbakhsh S (2011) An eye on the head: the development and evolution of craniofacial muscles. Development 138(12):2401–2415

Sassoon DA (1993) Myogenic regulatory factors: dissecting their role and regulation during vertebrate embryogenesis. Dev Biol 156(1):11–23

Scaal M, Bonafede A, Dathe V, Sachs M, Cann G, Christ B, Brand-Saberi B (1999) SF/HGF is a mediator between limb patterning and muscle development. Development 126(21):4885–4893

Schäfer K, Braun T (1999) Early specification of limb muscle precursor cells by the homeobox gene Lbx1h. Nat Genet 23(2):213–216

Scharner J, Zammit PS (2011) The muscle satellite cell at 50: the formative years. Skelet Muscle 1 (1):28

Shih HP, Gross MK, Kioussi C (2007) Cranial muscle defects of Pitx2 mutants result from specification defects in the first branchial arch. Proc Natl Acad Sci U S A 104(14):5907–5912

Shirozu M, Nakano T, Inazawa J, Tashiro K, Tada H, Shinohara T, Honjo T (1995) Structure and chromosomal localization of the human stromal cell-derived factor 1 (SDF1) gene. Genomics 28(3):495–500

Singh SK, Hawkins C, Clarke ID, Squire JA, Bayani J, Hide T, Henkelman RM, Cusimano MD, Dirks PB (2004) Identification of human brain tumour initiating cells. Nature 432(7015):396–401

Stebler J, Spieler D, Slanchev K, Molyneaux KA, Richter U, Cojocaru V, Tarabykin V, Wylie C, Kessel M, Raz E (2004) Primordial germ cell migration in the chick and mouse embryo: the role of the chemokine SDF-1/CXCL12. Dev Biol 272(2):351–361

Sun Y, Cheng Z, Ma L, Pei G (2002) Beta-arrestin2 is critically involved in CXCR4-mediated chemotaxis, and this is mediated by its enhancement of p38 MAPK activation. J Biol Chem 277(51):49212–49219

Sun Y, Wang J, Shelburne CE, Lopatin DE, Chinnaiyan AM, Rubin MA, Pienta KJ, Taichman RS (2003) Expression of CXCR4 and CXCL12 (SDF-1) in human prostate cancers (PCa) in vivo. J Cell Biochem 89(3):462–473

Tachibana K, Hirota S, Iizasa H, Yoshida H, Kawabata K, Kataoka Y, Kitamura Y, Matsushima K, Yoshida N, Nishikawa S, Kishimoto T, Nagasawa T (1998) The chemokine receptor CXCR4 is essential for vascularization of the gastrointestinal tract. Nature 393(6685):591–594

Tajbakhsh S, Rocancourt D, Cossu G, Buckingham M (1997) Redefining the genetic hierarchies controlling skeletal myogenesis: Pax-3 and Myf-5 act upstream of MyoD. Cell 89(1):127–138

Theis S, Patel K, Valasek P, Otto A, Pu Q, Harel I, Tzahor E, Tajbakhsh S, Christ B, Huang R (2010) The occipital lateral plate mesoderm is a novel source for vertebrate neck musculature. Development 137(17):2961–2971

Tzahor E (2014) Head Muscle Development. In: Brand-Saberi B (ed) Vertebrate myogenesis: stem cells and precursors, Results and problems in cell differentiation. Springer, Heidelberg

Tzahor E, Kempf H, Mootoosamy RC, Poon AC, Abzhanov A, Tabin CJ, Dietrich S, Lassar AB (2003) Antagonists of Wnt and BMP signaling promote the formation of vertebrate head muscle. Genes Dev 17(24):3087–3099

Valasek P, Evans DJR, Maina F, Grim M, Patel K (2005) A dual fate of the hindlimb muscle mass: cloacal/perineal musculature develops from leg muscle cells. Development 132(3):447–458

Valasek P, Theis S, DeLaurier A, Hinits Y, Luke GN, Otto AM, Minchin J, He L, Christ B, Brooks G, Sang H, Evans DJ, Logan M, Huang R, Patel K (2011) Cellular and molecular investigations into the development of the pectoral girdle. Dev Biol 357(1):108–116

Vasyutina E, Stebler J, Brand-Saberi B, Schulz S, Raz E, Birchmeier C (2005) CXCR4 and Gab1 cooperate to control the development of migrating muscle progenitor cells. Genes Dev 19 (18):2187–2198

Verbout AJ (1985) The development of the vertebral column. Adv Anat Embryol Cell Biol 90:1–122

Vila-Coro AJ, Rodríguez-Frade JM, Martín De Ana A, Moreno-Ortíz MC, Martínez-A C, Mellado M (1999) The chemokine SDF-1alpha triggers CXCR4 receptor dimerization and activates the JAK/STAT pathway. FASEB J 13(13):1699–1710

Weintraub H, Davis R, Tapscott S, Thayer M, Krause M, Benezra R, Blackwell TK, Turner D, Rupp R, Hollenberg S (1991) The myoD gene family: nodal point during specification of the muscle cell lineage. Science 251(4995):761–766

Williams PL, Bannister LH (eds) (1995) Gray's anatomy. The anatomical basis of medicine and surgery, 38th edn. Livingstone, New York, NY

Williams BA, Ordahl CP (1994) Pax-3 expression in segmental mesoderm marks early stages in myogenic cell specification. Development 120(4):785–796

Wilting J, Brand-Saberi B, Huang R, Zhi Q, Köntges G, Ordahl CP, Christ B (1995) Angiogenic potential of the avian somite. Dev Dyn 202(2):165–171

Wilting J, Schneider M, Papoutski M, Alitalo K, Christ B (2000) An avian model for studies of embryonic lymphangiogenesis. Lymphology 33(3):81–94

Wilting J, Papoutsi M, Othman-Hassan K, Rodriguez-Niedenführ M, Pröls F, Tomarev SI, Eichmann A (2001) Development of the avian lymphatic system. Microsc Res Tech 55 (2):81–91

Yusuf F, Brand-Saberi B (2006) The eventful somite: patterning, fate determination and cell division in the somite. Anat Embryol 211(Suppl 1):21–30

Yusuf F, Brand-Saberi B (2012) Myogenesis and muscle regeneration. Histochem Cell Biol 138 (2):187–199

Yusuf F, Rehimi R, Dai F, Brand-Saberi B (2005) Expression of chemokine receptor CXCR4 during chick embryo development. Anat Embryol 210(1):35–41

Yusuf F, Rehimi R, Moroşan-Puopolo G, Dai F, Zhang X, Brand-Saberi B (2006) Inhibitors of CXCR4 affect the migration and fate of CXCR4+ progenitors in the developing limb of chick embryos. Dev Dyn 235(11):3007–3015

Zammit PS (2008) All muscle satellite cells are equal, but are some more equal than others? J Cell Sci 121(Pt 18):2975–2982

Zou YR, Kottmann AH, Kuroda M, Taniuchi I, Littman DR (1998) Function of the chemokine receptor CXCR4 in haematopoiesis and in cerebellar development. Nature 393(6685):595–599

Hypaxial Muscle: Controversial Classification and Controversial Data?

Karl R. Wotton, Frank R. Schubert, and Susanne Dietrich

Abstract Hypaxial muscle is the anatomical term commonly used when referring to all the ventrally located musculature in the body of vertebrates, including muscles of the body wall and the limbs. Yet these muscles had very humble beginnings when vertebrates evolved from their chordate ancestors, and complex anatomical changes and changes in underlying gene regulatory networks occurred. This review summarises the current knowledge and controversies regarding the development and evolution of hypaxial muscles.

1 Introduction

Vertebrates evolved from chordate ancestors that lived in water (reviewed in (Clack 2002; Freitas et al. 2014). Their mode of movement was a side-to-side undulation of body and tail, which can still be seen in extant chordates, but also in aquatic and semi-aquatic vertebrates (exception: aquatic mammals; see below). This side-to-side undulation is facilitated by reiterated (segmented) blocks of muscle—the myotomes (Goodrich 1958). The myotomes work against a central skeletal element, initially the notochord, later the vertebral column. The myotomes are innervated by somatic motor neurons which are connected with activating and inhibitory interneurons such that muscle contracts in an alternating fashion on either side of the

K.R. Wotton
EMBL/CRG Systems Biology Research Unit, Centre for Genomic Regulation (CRG), Dr. Aiguader 88, 08003 Barcelona, Spain

Universitat Pompeu Fabra (UPF), Barcelona, Spain

F.R. Schubert
Institute for Biomedical and Biomolecular Science (IBBS), School of Biology, University of Portsmouth, King Henry Building, King Henry I Street, Portsmouth PO1 2DY, UK

S. Dietrich (✉)
Institute for Biomedical and Biomolecular Science (IBBS), School of Pharmacy and Biomedical Sciences, University of Portsmouth, St. Michael's Building, White Swan Road, Portsmouth PO1 2DT, UK
e-mail: susanne.dietrich@port.ac.uk

© Springer-Verlag Berlin Heidelberg 2015

B. Brand-Saberi (ed.), *Vertebrate Myogenesis*, Results and Problems in Cell Differentiation 56, DOI 10.1007/978-3-662-44608-9_2

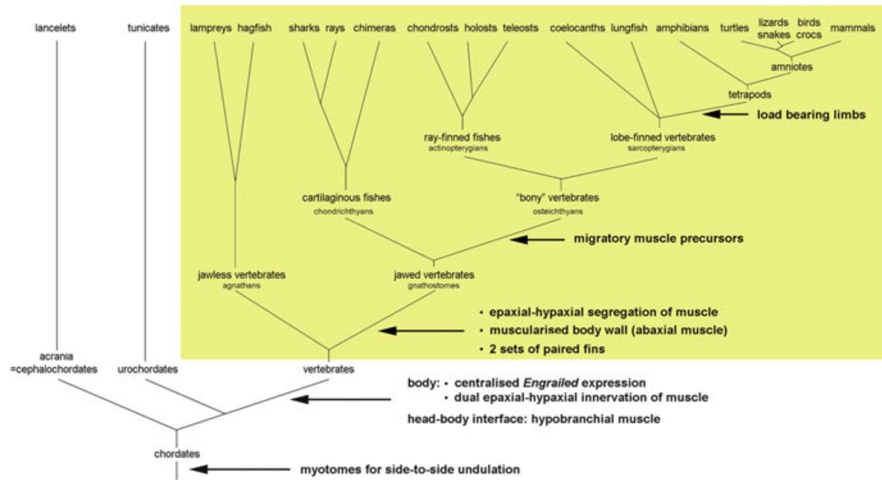


Fig. 1 Vertebrate phylogenetic tree, indicating the key changes in the organisation of their body musculature that underpinned changes in movement pattern. The basic chordate movement pattern is swimming via side-to-side undulations of the body and tail, relying on segmented muscle blocks—the myotomes—on either side of the body. The first steps towards three-dimensional mobility were taken in the shared ancestors of agnathans and gnathostomes, when *Engrailed* was recruited into the somite to facilitate the separate innervation of the dorsal/epaxial and ventral/hypaxial domain of the myotome. Likewise, in this ancestor the most rostral segments became incorporated into the head and muscle was deviated from a role in locomotion to role in pharyngeal support, respiration and food uptake. Specifically, the ventral/hypaxial muscle precursors were recruited to provide elaborate hypobranchial muscles. In the lineage leading to jawed vertebrates, epaxial–hypaxial muscle became fully segregated. Moreover, the lateral mesoderm developed two distinct leafs, facilitating the establishment of a muscularised body wall and the evolution of paired fins. Initially, muscle penetrated the outer, somatopleural aspect of the lateral mesoderm as a somitic outgrowth. Yet in the lineage leading to osteichthyans, a molecular program that allowed the de-epithelialisation and emigration of muscle precursors evolved. This program was first used to generate the muscles of the pectoral fins, but in the lineage leading to sarcopterygians, it was co-opted into the pelvic fins/hind limbs. It is thought that the resolution of segmental boundaries allowed the redistribution of muscle cells and, together with the more mobile insertion of the sarcopterygian fin/limb in the shoulder girdle, it facilitated the evolution of load-bearing limbs

body, and a wave of contractions runs from rostral to caudal. This creates a force against water as a viscous medium and propels the body forward (Kiehn 2011).

The myotomes were initially set up as dorsoventrally continuous half-rings (Goodrich 1958; Fetcho 1987). Yet in the ancestor of jawed vertebrates, muscle became split into distinct, separately innervated dorsal and ventral muscle blocks, which allowed full three-dimensional movements for the first time (Goodrich 1958; Fetcho 1987; Fig. 1). Moreover, when the lateral mesoderm evolved to form two distinct leaves, an inner splanchnopleura and an outer somatopleura, muscle penetrated the outer layer, thus leading to a muscularised body wall (Onimaru et al. 2011). Finally paired fins evolved. In most cartilaginous vertebrates (chondrichthyans) and

in ray-finned (actinopterygian) bony[1] vertebrates (osteichthyans), paired fins are mainly used for steering. Yet in lobe-finned animals (sarcopterygian osteichthyans), these fins eventually changed into load-bearing limbs that allowed the tetrapods to conquer land and to take to the air (Clack 2002). These amazing changes in body plans and locomotion took some 500 million years and allowed vertebrates to populate every ecological niche on Earth: land, air, fresh and marine waters. With that respect, vertebrates are one of the most successful animal group ever.

The anatomical changes that allowed the change of vertebrate movement patterns predominantly affected the lay-out of the ventral muscular system, traditionally referred to as "hypaxial", and this review will retrace their development and evolution. It also will discuss the "primaxial–abaxial" subdivision of muscle that is often portrayed as contrasting concept. Finally, the review will provide an overview of a specialised type of hypaxial muscle precursors that evolved in the osteichthyan lineage and that is thought to have aided the evolution of load-bearing limbs—the migratory hypaxial muscle precursors.

2 Developmental Anatomy of Dorso-Ventral Muscles and the Classical Epaxial–Hypaxial Concept

In all vertebrates, the skeletal musculature of the body and fins/limbs originates from the segmented paraxial (=next to the axial notochord) mesoderm termed somites, and muscle is laid down in waves (reviewed in: Bryson-Richardson and Currie 2008; Buckingham 2006; Fig. 2). The first muscle (primary myotomes) is immediately contractile. This is essential since anamniote vertebrates (as well as their chordate relatives) develop via free-feeding, motile larvae (Goodrich 1958). Yet, differentiated muscle fibres are postmitotic, thus limiting muscle growth to an increase in fibre size by hypertrophy. However, vertebrates have set aside muscle stem cells that drive hyperplastic muscle growth and muscle repair upon injury (reviewed in: Bryson-Richardson and Currie 2008; Buckingham 2006). These stem cells initially reside in a structure superficial to the myotome, the dermomyotome. However, eventually these cells populate the muscles, thereby establishing a resident pool of stem cells. In the adult, these cells are located underneath the basal lamina of muscle fibres and are referred to as satellite cells. In actinopterygians such as teleosts, muscle stem cells drive the continuous muscle growth typical for these animals. In amniotes, the satellite cells become quiescent and are only activated and proliferative upon injury.

In jawed vertebrates, the myotomes become segregated into dorsal and ventral components. This is achieved by the intercalation of a physical boundary, the

[1] The phrase "bony" vertebrate as a more colloquial term for osteichthyans is somewhat misleading since mineralised bone was already present in stem group gnathostomes and was secondarily lost in sharks and rays, (Zhu et al. 2013).

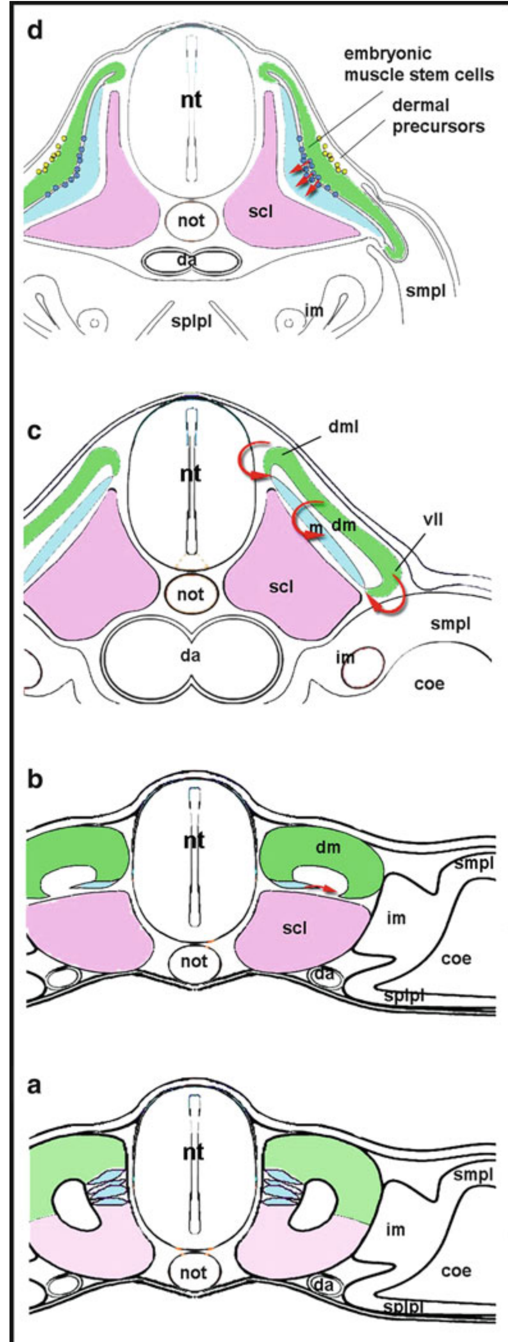


Fig. 2 Vertebrate muscle is generated in waves. Schematic cross sections of amniote flank somites, dorsal to the top, modelled after the chicken. In line with the rostrocaudal progression of somite formation and differentiation, the developmentally youngest somites are shown at the *bottom*, the most mature at the *top*. (**a**) Soon after the epithelial somite formed, its dorsal territory becomes specified as dermomyotome (*light green*), its ventral territory as sclerotome (*light pink*), and cells in the medial wall of the somite are specified as the first myogenic cells (*turquoise*). (**b**) The myogenic cells, also referred to as muscle pioneers, spread (*red arrow*) and form a scaffold between the now morphologically defined dermomyotome (*green*) and sclerotome (*pink*). (**c**) More cells are being added to the scaffold from the dorsomedial, ventrolateral, rostral and caudal edges of the dermomyotome (*red arrows*), leading to a morphologically well-defined, contractile myotome (*turquoise*). (**d**) Eventually, the dermomyotome de-epithelialises, releasing myogenic stem cells into the myotome (*red arrows*). These cells drive the foetal and perinatal growth of muscle and provide the adult muscle stem cells (satellite cells)

thoracolumbar fascia or horizontal myoseptum (Goodrich 1958; Gray 1995). In teleost fish, the dorsoventral subdivision of muscle occurs at an early time point and is organised by specialised, *engrailed* expressing slow muscle cells. These cells are somewhat misleadingly termed muscle pioneers since they express muscle structural genes at an early time point (Devoto et al. 1996; Currie and Ingham 1996). However, their key role is to serve as a first target for the axons of the three large, primary somatic motorneurons and organise the projection of one of them to the dorsal muscle block, one to the ventral muscle block, and one to innervate the slow-twitch muscle at the dorsoventral boundary (Beattie and Eisen 1997), reviewed by (Lewis and Eisen 2003). When *engrailed* function is disrupted, severe innervation defects occur (Ahmed et al. manuscript in preparation; Fig. 3).

In teleosts, eventually the smaller, secondary motorneurons outnumber the primary motorneurons and take over to drive muscle contraction (Fetcho 1987; Lewis and Eisen 2003). However, these neurons use the pre-existing scaffold for their axonal pathfinding. In amniotes in contrast, it is generally held that only secondary-type motorneurons are being used (Fetcho 1987). They are organised into two pools in the ventral spinal cord, with the medially located pool (medial motor column) destined to innervate the dorsal muscles, and the laterally located neurons (hypaxial motor column) innervating the ventral muscles (reviewed in: Tsuchida et al. 1994). Yet in all vertebrates, the physical segregation of muscle is matched by their separate innervation, such that the dorsal and ventral muscles can contract independently. According to their distinct innervation, muscles have classically been distinguished as epaxial (innervated by the medial motor column via the dorsal ramus of the spinal nerve) or hypaxial (innervated by the hypaxial motor column via the ventral ramus of the spinal nerve; Fig. 4a, Ai).

Interestingly, in amniotes, the dorsoventrally distinct innervation of body muscle occurs before the myotome is physically segregated (Tosney and Landmesser 1985; Tosney 1987; reviewed in: Fetcho 1987). Yet this innervation pattern also relies on *Engrailed* (*En1*; Ahmed et al., manuscript in preparation). *En1* is initially expressed in the central dermomyotome where it sets up a molecular and compartment boundary (Cheng et al. 2004). Expression of *En1* is brought into the myotome when the muscle stem cells arrive from the de-epithelialising dermomyotome (Ahmed et al. 2006). *En1* then supports the outgrowth of the dorsally projecting axons and suppresses the outgrowth of the ventrolaterally projecting axons (Ahmed et al., manuscript in preparation; Fig. 3). Thus, although *En1* function has shifted in time, it is remarkable that in jawed vertebrates, it is associated with epaxial–hypaxial boundary formation and innervation. Moreover, in all vertebrates investigated *Engrailed* expression is controlled by the Shh signalling molecule released from the notochord, suggesting the conservation of key parts of the underlying regulatory network (Cheng et al. 2004; Hammond et al. 2009; Maurya et al. 2011).

Jawless vertebrates such as the lamprey already have a dorsal and ventral innervation point of their myotome even though a horizontal myoseptum is absent (Fetcho 1987). Moreover, markers have been identified that distinguish the dorsal and the lateral edge of the somite, suggesting that the first steps towards an epaxial–hypaxial segregation of muscle had been taken before the agnathan-gnathostome

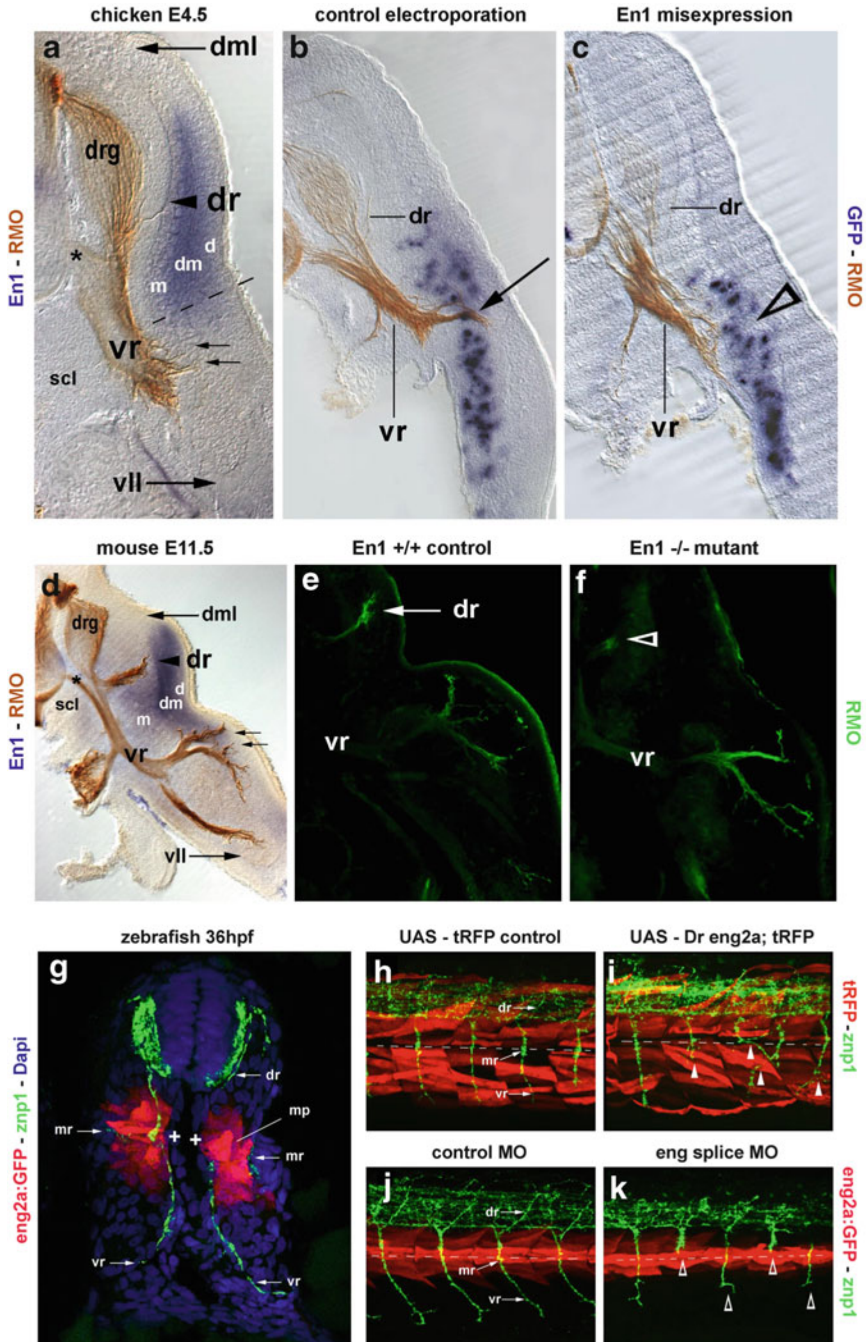


Fig. 3 Conserved role of *Engrailed* in organising the epaxial–hypaxial innervation of muscle. (**a**–**c**) Role of *En1* in the chicken embryo. (**a**) Cross sections of chicken flank somites at embryonic day E4.5 of development, dorsal to the *top*, medial to the *left*. *Engrailed 1* (*En1*) expression is shown in *blue*, intermediate neurofilaments of nerve axons are revealed with the RMO antibody in

divide (Kusakabe et al. 2011). Interestingly, also in the lamprey, one of its *engrailed* genes is expressed in the centre of the myotome, molecularly separating its dorsal and ventral aspect (Hammond et al. 2009; Matsuura et al. 2008). This suggests that already before the agnathan-gnathostome divide, *eng*/*En* had been

Fig. 3 (continued) brown. Note that *En1* expressing cells are leaving the central dermomyotome and populate the central myotome underneath. The developing ventral ramus of the spinal nerve navigates around the *En1* expression domain and targets the hypaxial myotome; first contact with the myotome is made when axons of the cutaneous branch of the ventral ramus travel along the ventral boundary of the *En1* domain and project towards the dermis (*small arrows*). The dorsal ramus lags developmentally behind; its axons target the *En1* domain (*arrowhead*). (**b**–**c**) Gain-of-function experiment; phenotype displayed on cross sections. Flank somites were electroporated at E2.5 with (**b**) a *GFP* expressing control construct or (**c**) a bi-cistronic *GFP* and *En1* expressing experimental construct. 24 h later, expression of the construct was revealed by in situ hybridisation in *blue* and the position of the axons by RMO staining in *brown*. Note that in the *En1* misexpressing somites, the ventral ramus of the spinal nerve defasciculated and failed to form the cutaneous branch of this ramus (*open arrowhead*). (**d**–**f**) Role of *En1* in the mouse embryo. (**d**) Cross sections of mouse flank somites at E11.5 of development, dorsal to the *top*, medial to the *left*; *En1* expression in *blue*, RMO staining in *brown*. Note that marker gene expression and axonal projections in the mouse closely match that of the chicken. (**e**–**f**) Loss-of-function experiment, axons were revealed by RMO antibody staining on cross sections (green staining). Note that in wildtype litter mates, the dorsal ramus of the spinal nerve is well developed and innervates the epaxial myotome (**e**). In *En1* deficient embryos, that dorsal ramus falls short of its target (**f**, *open arrowhead*). (**g**–**k**) Role of *engrailed* genes in the zebrafish. (**g**) Cross section of a 36hpf zebrafish eng2a:GFP embryo. *engrailed* expression as revealed by the GFP transgene expression is shown in *red*; axons are stained with the znp1 antibody (*green*) and cell nuclei with Dapi (*blue*). Initially, the primary motor neurons all project to the *eng* expressing muscle pioneers (+, mp) which organise the formation of the horizontal myoseptum. Subsequently, the primary and accompanying secondary motor neurons located next to the posterior somite half extend their axons ventrally to contribute to the ventral ramus of the spinal nerve and to innervate the hypaxial myotome. Motor neurons neighbouring the anterior somite half send their axons laterally along the developing horizontal myoseptum to form the fish-specific medial ramus and to target the superficial slow muscles. Motor neurons in the middle of each segment withdraw their connection to the muscle pioneers and project dorsally to form the dorsal ramus and to innervate the epaxial myotome. (**h**–**k**) Lateral views of 36hpf experimental zebrafish embryos, anterior to the *left*. The position of the developing horizontal myoseptum is indicated by a stippled line. (**h**–**i**) Gain-of-function experiment: Transgenic α actin-Gal4 embryos were used to drive expression of (H) a UAS-tRFP control construct or (**i**) a bi-cistronic construct encoding tRFP as well as zebrafish engrailed 2a. Cells expressing the constructs fluoresce in red; axons are revealed with the znp1 antibody in *green*. Note that *engrailed* misexpression leads to severe misguidance of motor axons (**i**, *arrowheads*). (**j**–**k**) Loss-of-function experiment: eng2a:GFP embryos (transgene expression shown in red, znp1-stained axons in green as in (**g**) were treated with (**j**) a control morpholino or (**k**) a morpholino cocktail targeting the splice sites of *engrailed1a*, *1b* and *2a* which are all expressed in muscle pioneers. Note that this knock down of *eng* function blocked axonal outgrowth, and axons stalled or took up erratic paths in search for their targets (**k**, *open arrowheads*). The data shown here are the work of Mohi U. Ahmed, Ashish K. Maurya, Louise Cheng, Erika C. Jorge, Frank R. Schubert, Pascal Maire, M. Albert Basson, Philip W. Ingham and Susanne Dietrich. *d* dermis precursors, *dm* dermomyotome, *dml* dorsomedial lip of the dermomyotome, *dr* dorsal ramus of the spinal nerve, *drg* dorsal root ganglion, *m* myotome, *mp* muscle pioneers, *mr* medial ramus of the spinal nerve, *scl* sclerotome, *vll* ventrolateral lip of the dermomyotome, *vr* ventral ramus of the spinal nerve. The *asterisk* in (**a**,**d**) marks the axons of motor neurons projecting out of the neural tube

recruited into the developing musculature where it paved the way for the dorso-ventral segregation and innervation of muscle and the evolution of full three-dimensional mobility. On the other hand, when mammals returned to the sea, they adapted to movement in water with fully segregated epaxial–hypaxial muscle in place. It can be speculated that this divide was the basis to evolve upwards-downwards undulations of the body and tail as seen best in dolphins and whales.

3 The Primaxial–Abaxial Concept and the Lateral Somitic Frontier

Skeletal muscle fibres are organised into anatomically defined muscles via several layers of connective tissue (Gray 1995). Moreover, muscle can only fulfil its role when anchored on skeletal elements via tendons or aponeuroses. Thus, functional muscle has an intricate relationship with the various types of connective tissue. Indeed, even though initially muscle and connective tissue develop independently, eventually both tissues rely on each other for function and survival (Murphy et al. 2011). Interestingly, in muscle-less limbs, connective tissue organises itself in the correct anatomical pattern, anticipating the position of muscle (Kardon et al. 2003). This indicates that the connective tissue directs the muscle cells to their defined places. Yet at different locations in the body, connective tissue is made from different cell types, and hence, muscle has to adjust to different partners. This has led to the primaxial–abaxial concept of muscle development (Fig. 4b,Bi).

Epaxial muscles, but also some hypaxial muscles—for example the sub-vertebral muscle of the neck and the muscles associates with the ribs—receive their connective tissue from the somites. Thus, the cells always remain in a paraxial—or primaxial—environment. On the other hand, hypaxial muscle precursors for muscles associated with the sternum, the body wall or the limbs enter a new environment, namely the dorsal leaf of the lateral mesoderm (somatopleura), and all the connective tissue is derived from this environment (Durland et al. 2008). When heterotopically grafted, muscle precursors entering the lateral plate environment switch *Hox* gene expression to match the position values found on site (Nowicki and Burke 2000). Thus, these hypaxial cells cross a boundary, termed "lateral somitic frontier". They will settle far from their original position and are patterned by their new environment, and hence have been termed "abaxial" (reviewed in: Shearman and Burke 2009). In Amphioxus and in the lamprey, the lateral mesoderm of the body does not split into splanchnic and somatic lateral mesoderm, and muscle does not enter this environment (Onimaru et al. 2011; Tulenko et al. 2013). Thus, abaxial body muscle, i.e. a muscularised body wall and derivates thereof (see below) may be a novelty that emerged in the lineage which eventually led to the jawed vertebrates (Fig. 1). How connective tissue cells communicate their positional values to muscle cells is not clear. It has been shown, however, that homeodomain transcription factors can act as short range signalling

molecules, both in vertebrates and in insects (reviewed in: Brunet et al. 2007; Layalle et al. 2011). Thus, it is tempting to speculate that a similar process may allow the alignment of *Hox* gene expression patterns. However, single cells versus group cell grafting performed in a cranial environment suggested that, if the grafted cells have enough neighbours of their own kind, they retain their original Hox code (Trainor and Krumlauf 2000). Thus, more work is needed to determine cell–cell communication at the lateral somitic frontier. Yet, it should be emphasized that the classical epaxial–hypaxial concept and the primaxial–abaxial concept are not necessarily exclusive; they simply refer to different aspects of ventrolateral muscle development (Fig. 4).

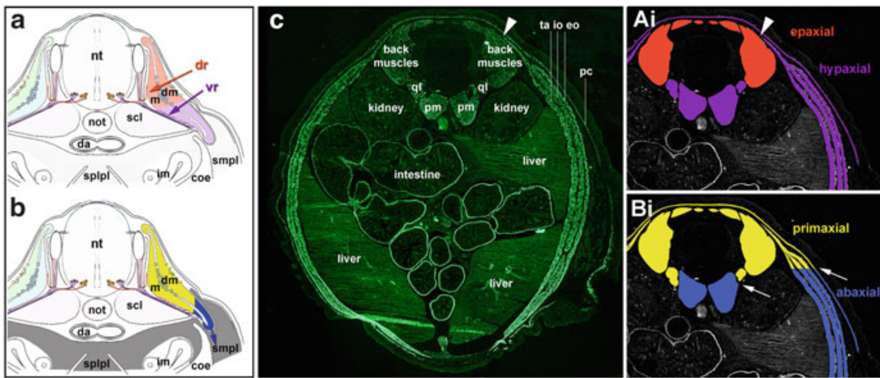


Fig. 4 Comparison of the epaxial–hypaxial and primaxial–abaxial concept. (**a**) Schematic cross section of an amniote abdominal somite at the time of innervation, modelled after the section shown in Fig. 4a. Motor neurons located in the medial motor column and contributing to the dorsal ramus of the spinal nerve are shown in orange. Motor neurons located in the hypaxial motor column and contributing to the ventral ramus of the spinal nerve are shown in purple. Areas of the somite targeted by the dorsal or ventral ramus of the spinal nerve are displayed in matching colours. Notably, the myotome is still dorsoventrally continuous at this stage. (**b**) Same schematic cross section as in (**a**). Areas of the somite developing in association with the sclerotome-derived axial skeleton are shown in *yellow*, areas of the somite that grow out into the somatopleural leaf of the lateral mesoderm are shown in *blue*. The direction of outgrowth is marked by a *blue arrow*. The lateral mesoderm is shown in *grey*. (**c**) Cross section of the abdomen of a newborn mouse, muscle is stained for skeletal muscle Myosin in green. The *arrowhead* indicates the insertion of the body wall muscles at the thoracolumbar fascia. (Ai) Cross section as in (C), with muscles colour-coded according to their innervation as in (**a**). Muscles innervated by the dorsal ramus of the spinal nerve are the epaxial muscles (*red*). Muscles innervated by the ventral ramus are the hypaxial muscles (*purple*). (Bi) Cross section as in (**c**); the arrows indicates the boundary between sclerotome and lateral mesoderm derived connective tissue as revealed by the lineage tracing of *Prx1*-expressing cells (Durland et al. 2008). Muscles developing in a sclerotome-derived environment are colour-coded *yellow* as in (B). These are the primaxial muscles. Muscles developing in a somatopleura-derived environment are colour-coded *blue* as in (**b**). These are the abaxial muscles. *coe* coelomic cavity, *da* dorsal aorta, *dm* dermomyotome, *dr* dorsal ramus of the spinal nerve, *eo* external oblique muscle, *im* intermediate mesoderm, *io* internal oblique muscle, *m* myotome, *nt* neural tube, *not* notochord, *pc* panniculus carnosus muscle, *pm* psoas muscle, *ql* quadratus lumborum muscle, *scl* sclerotome, *smpl* somatopleural leaf of the lateral mesoderm, *splpl* splanchnopleural leaf of the lateral mesoderm, *ta* transversus abdominis muscle, *vr* ventral ramus of the spinal nerve

4 Migratory Muscle Precursors for the Paired Fins and Limbs: An Osteichthyan Innovation

In tetrapods, even though the lateral swaying of the body is still an important part in the movement of amphibians and reptiles, locomotion clearly relies on load-bearing limbs and their associated musculature. Innervated by the lateral motor column a specialist group of hypaxial motor neurons only found at limb levels reviewed in Murakami and Tanaka 2011 and developing in the lateral mesoderm that provides the limb connective tissue and the limb skeleton, limb muscles are both hypaxial and abaxial. Embryological studies in the chicken established that in amniotes, limb muscles develop from cells that detach from the lateral lip of the dermomyotome and actively migrate into the limb, where they become organised into dorsal and ventral muscles masses to give rise to the extensor and flexor muscles groups, respectively (Chevallier et al. 1977; Christ et al. 1977; Hayashi and Ozawa 1995; Fig. 5a). Painstaking histological and lineage tracing experiments in various fish species showed that muscle precursors undertaking long-range migration muscularise the pectoral fins of teleosts (ray-finned "bony" vertebrate), while in cartilaginous vertebrates, somites form extensions that reach into the fin anlage in a similar fashion as they grow out into the body wall to form abdominal muscles (Neyt et al. 2000). The pelvic fins of lungfish (a lobe-finned relative of tetrapods), teleosts and paddlefish (both ray-finned osteichthyans) receive muscle precursors from somitic extensions that, when close to their target site, deepithelialise to release individual cells. The pelvic fin muscles of sharks and chimeras are made in the same way as their pectoral musculature, namely from somitic extensions (Cole et al. 2011). This has led to the view that hypaxial/abaxial muscle formation via somitic extensions is the evolutionarily older mechanism of muscle delivery, while migratory muscle precursors evolved later in the lineage leading to osteichthyans. They were first established for the pectoral fin/forelimb and subsequently for the pelvic fin/hind limb. In line with this view, a molecular program has been deciphered that specifically operates at teleost pectoral fin levels/tetrapod limb levels, and acts on top of the generic program for hypaxial myogenesis (Fig. 5b).

The generic program for hypaxial myogenesis is best researched in amniotes. Here, the lateral somite domain is specified by Bmp4 released from the lateral mesoderm (Pourquié et al. 1996). Together with Wnt signals from the surface ectoderm, Bmp upregulates the expression of the pre-myogenic gene *Pax3* (Dietrich et al. 1998; Fan et al. 1997; Tajbakhsh et al. 1998). Six transcription factors, when activated by their Eya partners, contribute to the upregulation of *Pax3*, and Pax3 enhances its own expression. Together, Six and Pax3 transcription factors facilitate the generation of hypaxial skeletal muscle cells (Tremblay et al. 1998; Borycki et al. 1999; Grifone et al. 2005; Grifone et al. 2007). This occurs after Bmp4 cooperated with Notch signalling to facilitate the release of smooth muscle and endothelial precursors (Ben-Yair and Kalcheim 2008).

At limb levels, muscle precursors destined to emigrate express the homeodomain transcriptional repressor *Lbx1*, and in animals as diverse as teleosts

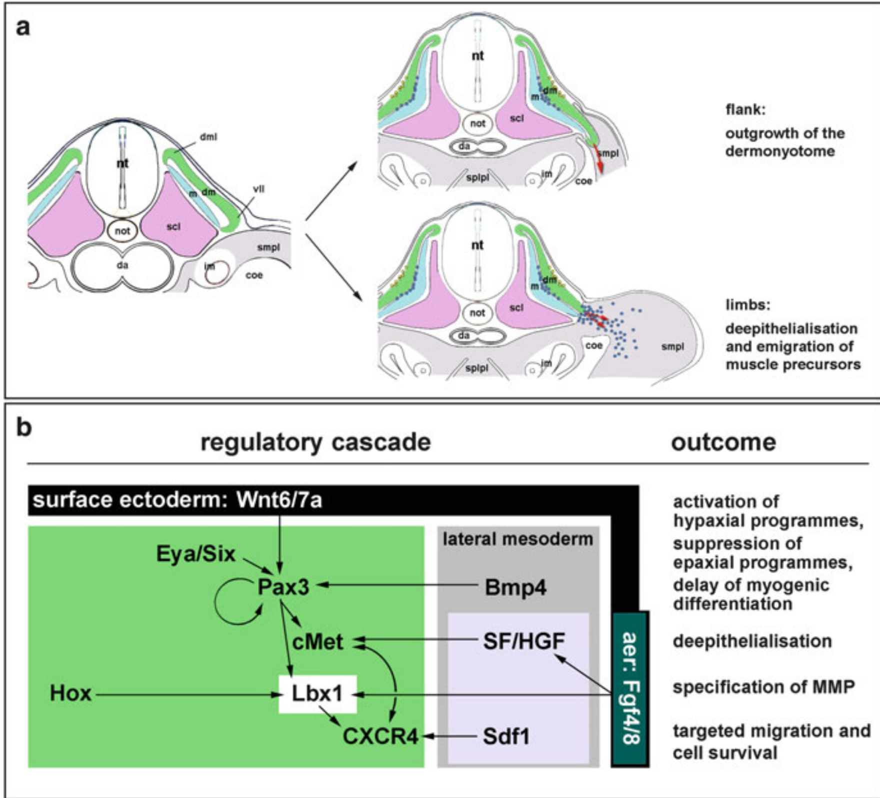


Fig. 5 The two modes of hypaxial muscle delivery—somitic outgrowth and targeted migration. (**a**) Schematic representation of maturing amniote somites at flank (*top*) and limb (*bottom levels*). At flank levels, the ventrolateral lip of the dermomyotome (vll) penetrates the somatopleura as a sheet. On its way, it deposits muscle precursors, thereby ensuring concomitant outgrowth of the myotome. At limb levels, the vll de-epithelialises, and cells actively migrate into the periphery. An intermediate mode of hypaxial muscle delivery is found in the teleost pelvic fins, where the vll of the outgrowing segment deepithelialises when the destination is reached. A similar mechanism has been reported for the formation of the ventralmost amniote abdominal muscle, the rectus abdominis. (**b**) Gene regulatory network for hypaxial muscle development. A generic program starting with Wnt signals from the surface ectoderm and Bmp signals from the lateral mesoderm operates at all axial levels. It upregulates the premyogenic gene *Pax3* which, together with premyogenic factors of the Six family, facilitates the generation of hypaxial myogenic cells. At limb levels, this generic program of hypaxial muscle formation is in operation. Yet, additional localised factors control the formation of migratory muscle precursors. Firstly, *Hox* gene expression in limb levels somites instructs these somites to activate the program of migratory rather than non-migratory muscle precursors (Alvares et al. 2003). In this context, Pax3 activates the marker for migratory muscle precursors, *Lbx1*, which in turn activates the cytokine receptor CXCR4 (Dietrich et al. 1999; Mennerich et al. 1998; Odemis et al. 2005; Vasyutina et al. 2005). Secondly, the limb lateral mesoderm provides the cMet ligand Scatter Factor/Hepatocyte Growth Factor (SF/HGF) and the CXCR4 ligand Sdf1 (Bladt et al. 1995; Prunotto et al. 2004; Vasyutina et al. 2005). Both signalling systems cooperate to control lip deepithelialisation, targeted cell migration and cell survival. Thirdly, the limb apical ectodermal ridge (aer) releases Fgf signalling molecules which are required for the expression of SF/HGF (Scaal et al. 1999). Importantly, Fgf molecules by themselves can override the program for non-migratory hypaxial muscle precursors, trigger *Lbx1* expression and serve as chemoattractants, thus ensuring that cells from the paraxial territory

(actinopterygians), lungfish and tetrapods (sarcopterygians), *Lbx1* (teleosts: *lbx1a, b*) is the bona fide marker for migratory muscle precursors (Cole et al. 2011; Dietrich 1999; Dietrich et al. 1999; Jagla et al. 1995; Martin and Harland 2006; Ochi and Westerfield 2009; Thisse et al. 2004); Figs. 5b and 6a, b, d and 7). Absence of *Lbx1* or misexpression of a dominant negative *Lbx1* construct prevents cell emigration into the paired fins/limbs, and only a subset of forelimb flexor muscle at the ventral junction to the limbs develops under the influence of local cues (Schäfer and Braun 1999; Gross et al. 2000; Brohmann et al. 2000; Ochi and Westerfield 2009; Martin and Harland 2006; Lours-Calet et al. 2014); Fig. 6f,Fi). Given this important role of *Lbx1*, the question of migratory muscle precursor formation has frequently been seen as a problem of localised *Lbx1* induction.

The mouse mutant *Splotch* is a well known model for muscle-less limbs (Franz et al. 1993; Bober et al. 1994; Tremblay et al. 1998), and in this animal, *Lbx1* expression is lost (Dietrich et al. 1999; Mennerich et al. 1998). *Splotch* mice carry a mutation for the paired box transcription factor *Pax3*, yet *Pax3* is expressed in the early somite, in the dermomyotome of more mature somites and is upregulated in the dorsomedial and ventrolateral dermomyotomal lips of all somites along the rostrocaudal body axis. Thus, while *Pax3* is necessary for *Lbx1* expression, it is not sufficient to position expression in limb-level somites. Interestingly, experiments in the zebrafish indicated that here, the duplicated *pax3b* gene had its expression restricted to pectoral fin somites and is required for *lbx* expression (*lbx2* in this case; Minchin et al. 2013). Thus, while displaying a variation on the theme, it suggests that the relationship of *Pax3* and *Lbx* is evolutionarily ancient.

It is well established that heterotopic transplantation of limbs or limb induction via implantation of Fgf beads in the flank of chicken embryos lead to the development of a muscularised and fully innervated ectopic limb (Chevallier et al. 1977; Christ et al. 1977; Hayashi and Ozawa 1995; Cohn et al. 1995). Moreover, the ectopic limb, its apical ectodermal ridge (aer) or the Fgf4/8 signalling molecule produced by the aer, all induce somitic *Lbx1* expression and the emigration of muscle precursors (Alvares et al. 2003). Furthermore, regulated by FGF from the aer, the limb mesenchyme produces the signalling molecule Scatter factor/Hepatocyte growth factor (Scaal et al. 1999). Its receptor cMet is found in all ventrolateral dermomyotomal lips, but the local activation of cMet leads to local lip deepithelialisation only (Bladt et al. 1995; Prunotto et al. 2004). Similarly, the cytokine Sdf1 which assists SF/HGF is expressed in the limb mesenchyme, and its CXCR4 receptor in the somitic dermomyotome (Odemis et al. 2005; Vasyutina et al. 2005). Together, this has led to the view that the limb overrides any pre-existing programme and is the key inducer of migratory muscle precursors.

Fig. 5 (continued) are recruited into the limb (Alvares et al. 2003). *coe* coelomic cavity, *da* dorsal aorta, *dm* dermomyotome, *im* intermediate mesoderm, *m* myotome, *MMP* migratory muscle precursors, *nt* neural tube, *not* notochord, *scl* sclerotome, *smpl* somatopleural leaf of the lateral mesoderm, *splpl* splanchnopleural leaf of the lateral mesoderm

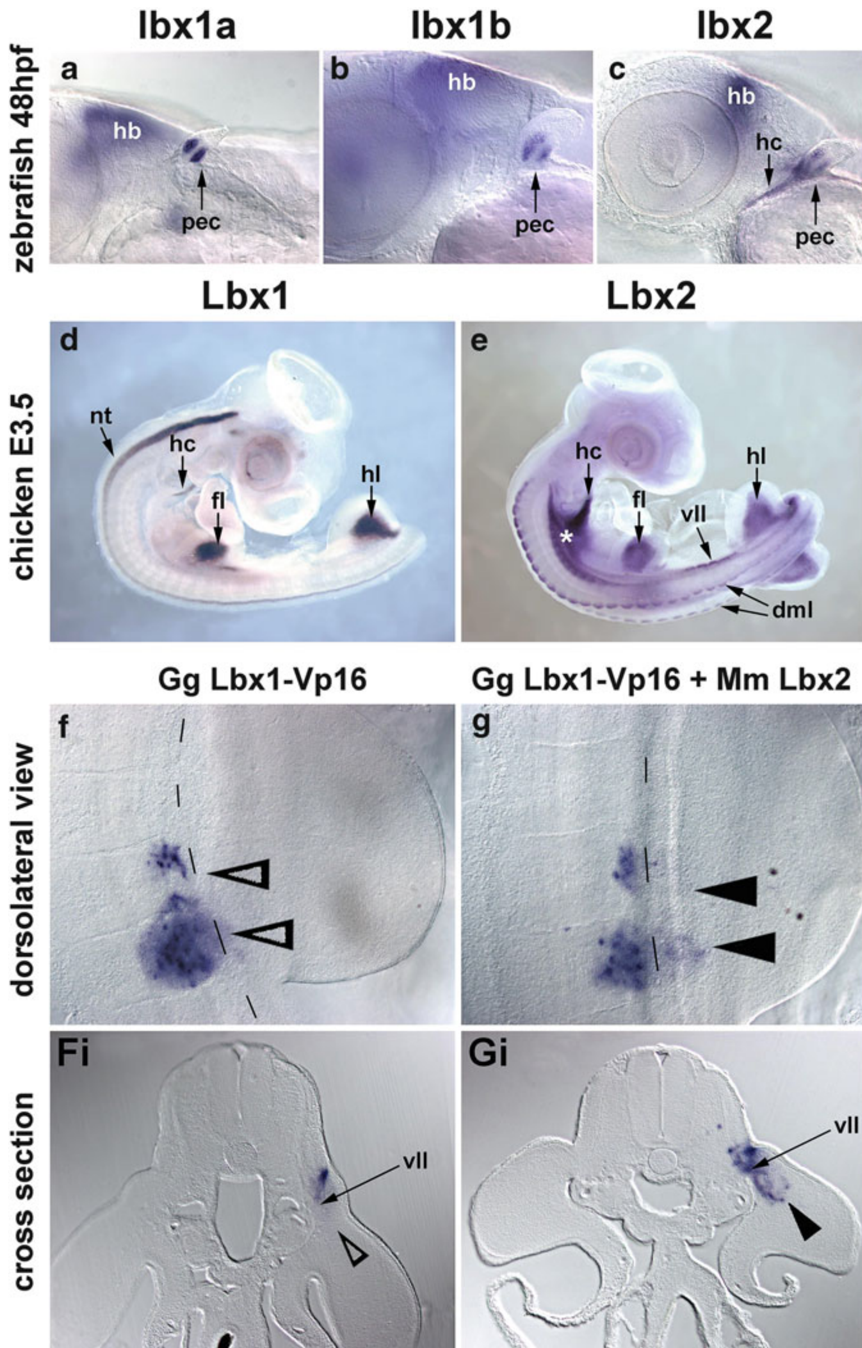


Fig. 6 Expression and function of *Lbx* genes. (**a**–**c**) Lateral views of zebrafish embryos, 48 h post-fertilisation (hpf), dorsal to the top, rostral to the *left*. Note that *lbx1a*, *lbx1b* and *lbx2* are all expressed in muscle precursors that have migrated into the pectoral fin. All are also expressed in the hindbrain (albeit *lbx2* in a smaller territory). Furthermore, *lbx2* is expressed in hypobranchial

However, evidence has accumulated that suggests the role of the limb has been overrated, and a more complex gene regulatory network has emerged (Fig. 5b).

A detailed characterisation of ectopic limbs revealed that the limb is not able to fully reprogram flank tissues since it does not force the spinal cord to generate a lateral motor column. Instead, the ectopic limb deviates axons originating from the flank hypaxial motor column to innervate the ectopic limb muscle rather than its normal target, the body wall musculature (Turney et al. 2003). Secondly, in cMet and SF/HGF mutants, while muscle precursors de-epithelialisation and emigration fails, *Lbx1* is well expressed (Dietrich et al. 1999), and in migratory muscle precursors, expression of CXCR4 is downstream of *Lbx1* (Vasyutina et al. 2005). This indicates that the specification of migratory muscle precursors is independent from cell de-epithelisation. Thirdly and most importantly, *Lbx1* expressing muscle precursors develop when competent, limb-level somites are exposed to any type of lateral mesoderm, including lateral mesoderm from the flank (Alvares et al. 2003). When *Hox* genes were misexpressed to switch the axial identity of flank somites to that of limb somites, these somites faithfully expressed *Lbx1* (Alvares et al. 2003; Fig. 5b). This indicates that intrinsic, Hox-dependent cues predispose somites towards either generating non-migratory or migratory muscle precursors.

5 Migratory Muscle Precursors for the Paired Fins and Limbs: A Vertebrate Innovation?

In osteichthyans, *Lbx1* genes are exclusively expressed in cells detaching and migrating away from the somites (Cole et al. 2011; Dietrich 1999; Dietrich et al. 1999; Jagla et al. 1995; Martin and Harland 2006; Ochi and Westerfield 2009; Thisse et al. 2004); this article; Fig. 7). Possibly the most extreme case is

Fig. 6 (continued) muscle precursors coalescing in the hypoglossal cord (hc) and temporarily in the dorsal and ventral tips of the myotome (not shown). (**d**, **e**) Lateral views of chicken embryos at embryonic day E3.5 days of development, rostral to the *top-right*. (**d**) *Lbx1*, in addition to its expression in the neural tube, is expressed in the migratory muscle precursors populating the limbs and the hypoglossal cord. *Lbx2* is not expressed in neural tissues, but shows a widespread expression in myogenic cells including muscle precursors in all ventrolateral dermomyotomal lips (migratory and non-migratory), muscle precursors in all dorsomedial lips and the myogenic neck lateral mesoderm (asterisk). (**f**, **g**) Dorsolateral views and (Fi,Gi) corresponding cross sections of electroporated chicken somites at forelimb levels. (**f**, Fi) Misexpression of a dominant negative chicken *Lbx1* construct (Gg Lbx1-Vp16; blue staining) interferes with the emigration of muscle precursors into the forelimb (*open arrowheads*). (**g**, Gi) Co-expression of the dominant negative construct (blue staining) together with mouse (Mm) *Lbx2* rescues muscle precursor emigration even though Mm *Lbx2* is divergent and not expressed in myogenic cells. The data shown here are the work of Karl Wotton and Susanne Dietrich. *dml* dorsomedial lip of the dermomyotome, *fl* fore limb, *hb* hind brain, *hc* hypoglossal cord, *hl* hind limb, *nt* neural tube, *pec* pectoral fin, *vll* ventrolateral lip of the dermomyotome

	***lbx/Lbx1*-type genes**					***Lbx2*-type genes**				
Species	**Gene**	**Expression in:**			**Reference**	**Gene**	**Expression in:**			**Reference**
		all dml	all vll	vll producing MMP only			all dml	all vll	vll producing MMP only	
Lamprey	*lbx-a* *		✓		(Kusakabe et al. 2011)					
Zebrafish	*lbx1a*			✓	(Ochi and Westerfield 2009); this article					
Zebrafish	*lbx1b*			✓	(Thisse et al. 2004); this article	*lbx2*	✓	✓		(Neyt et al. 2000; Ochi and Westerfield 2009); this article
Xenopus	*lbx1*		✓ **	✓ **	(Martin and Harland 2006)		No *lbx2* gene in the Xenopus genome assembly			(Wotton et al. 2008)
Chicken	*Lbx1*			✓	(Dietrich et al. 1998)	*Lbx2*	✓	✓		(Kanamoto et al. 2006); this article
Mouse	*Lbx1*			✓	(Jagla et al. 1995; Dietrich et al. 1999)	*Lbx2*	Not expressed in somites			(Chen et al. 1999)

* Phylogenetic analyses did not fully resolve whether the lamprey *lbx-A* gene is an ortholog of gnathostome *Lbx1*, or whether the gene arose before the two rounds (teleosts: three rounds) of gnathostome genome duplication and hence, would be a homologue of both, gnathostome *Lbx1* and *Lbx2* (Kusakabe et al. 2011; Wotton et al. 2008).

** Frog body wall muscles seem to form from migratory cells (Martin and Harland 2006).

Fig. 7 Presence and myogenic expression of vertebrate *Lbx* genes
Extant gnathostomes show evidence of 2 rounds of whole genome duplication during evolution, with additional lineages, including that of the teleosts, undergoing a third. Yet, owing to the early loss of duplicates, gnathostomes genomes only have a *Lbx1* gene (teleosts: *lbx1a* and *1b*) and a *Lbx2* gene; frogs may have lost their *lbx2*. Of these, *Lbx1* genes are almost invariably associated with migratory muscle precursors. *Lbx2* genes have a more widespread expression, labelling the dorsomedial as well as ventrolateral lips. The exceptions are mammals that have lost somitic *Lbx2* expression.
It is currently controversial whether the aforementioned genome duplications occurred before or after the gnathostome-agnathan split, whether an independent genome duplication occurred in agnathans, or whether in the agnathan lineage numerous individual genes were duplicated. Thus, the phylogenetic relationship of the only lamprey *lbx* gene identified so far, *lbx-A*, is unclear. Yet it is remarkable that this gene is expressed in all vll's along the body.
dml dorsomedial lips of the dermomyotome, *MMP* migratory muscle precursors, *vll* ventrolateral lips of the dermomyotome

Xenopus laevis, where body muscle is made from *lbx1* expressing muscle precursors that detach from the lateral lip edge of the somite (Martin and Harland 2001, 2006). Yet, the lamprey *lbx* homologue is expressed along the ventrolateral lip of all somites (Kusakabe et al. 2011). This raised the question whether the program for migratory muscle precursors arose much earlier than previously anticipated. Indeed, it has been suggested that when the somatopleura evolved as a separate layer of lateral mesoderm, it recruited programmes previously used to generate the dorsal and ventral fins—which are present already in agnathans (Freitas et al. 2006; reviewed in Freitas et al. 2014). Likewise, marker gene expression in the dorsomedial and ventrolateral tips of somites suggested that specific somitic programmes originally used to supply the musculature of those fins were established at this stage. Notably, the list of markers expressed in both tips includes *cMet* and the *Lbx1* paralog *Lbx2* (Neyt et al. 2000; Ochi and Westerfield 2009; Kanamoto et al. 2006; Yang et al. 1996; Fig. 6c, e and Fig. 7), exceptions are the mouse which has shed somitic *Lbx2* expression (Chen et al. 1999) and perhaps the frog for which no *lbx2* gene has been identified in the genome (Wotton et al. 2008). In zebrafish, knockdown of *lbx2* interferes with pectoral fin muscle development (Ochi and Westerfield 2009), and more amazingly, when a dominant negative form of *Lbx1* is introduced into limb-level chicken somites, muscle precursor migration can be rescued by co-expressing mouse *Lbx2* (K. Wotton and S. Dietrich, unpublished observations, Fig. 6g,Gi). This suggests that possibly already in agnathans, a programme utilising *lbx* genes may have been present at the dorsal and ventral extremes of somites that allowed their local dissipation and the release of cells into the dorsal and ventral fins. Yet, evidence is inconclusive: phylogenetic studies have not yet established the relationship of the lamprey *lbx* gene with the two gnathostome *Lbx* paralogs that arose from two rounds of genome duplication and subsequent gene loss (Wotton et al. 2008; Kusakabe et al. 2011). Moreover, careful functional studies on gnathostome *Lbx1* genes pointed at roles in controlling precursor cell proliferation and suppressing premature differentiation both in development and in activated satellite cells, rather than roles specific to cell migration (Mennerich and Braun 2001; Martin and Harland 2006; Watanabe et al. 2007). Finally, already in the protostome *Drosophila melanogaster*, ladybird/lbx function is associated with myogenesis (Jagla et al. 1998). Thus, gnathostome *Lbx* genes may be rather overrated as markers and may simply have a generic association with the generation of muscle precursors.

6 Hitching a Ride: The Development and Evolution of Hypopharyngeal and Tongue Muscle

During vertebrate evolution, the most rostral (occipital) somites were incorporated into the head (Gans and Northcutt 1983). Their sclerotomes were recruited to accommodate for larger brain sizes by reinforcing the base of the skull, and a

proportion of muscle precursors were deviated from making muscle for locomotion. Instead, these cells were recruited to contribute to the caudal pharyngeal arches and to provide an elaborate hypopharyngeal muscular system, all crucial in ventilating the gills and in food uptake (Goodrich 1958), recently reviewed in (Sambasivan et al. 2011). In lung-breathing tetrapods, the pharyngeal arches are not required for respiration any more, but the role in particular of the hypopharyngeal and, as an important component, the tongue muscles, remained crucial. Interestingly, in osteichthyans the hypopharyngeal/tongue muscle precursors undertake long-range migration, and they all express *Lbx1* (Dietrich et al. 1999; Lours-Calet et al. 2014; Martin and Harland 2006). Yet, in mouse mutants for the upstream regulator *Pax3*, in *cMet* and *SF*/*HGF* mutants as well as in *Lbx1* mutants, hypobranchial muscle formation is reduced, not abolished (Bladt et al. 1995; Prunotto et al. 2004; Schäfer and Braun 1999; Gross et al. 2000; Brohmann et al. 2000; Lours-Calet et al. 2014). Similarly, misexpression of a dominant negative *Lbx1* construct in occipital somites in the chicken only delayed the formation of hypopharyngeal muscle (Lours-Calet et al. 2014). Likewise, when somites normally producing non-migratory muscle precursors only, or head mesoderm that is unable to read signals for somitic myogenesis, were grafted in place of the somites normally providing hypobranchial muscle, the grafts contributed cells to this musculature (Mackenzie et al. 1998; Lours-Calet et al. 2014). Finally, the hypopharyngeal musculature of the lamprey is thought to derive from somitic extensions, not migratory cells (Goodrich 1958). Together, this suggests that there is an alternative, evolutionarily ancestral mechanism of hypopharyngeal muscle precursor transport that does not require active migration. Intriguingly, molecular markers for all occipital tissues and cells types extend their expression along the floor of the pharynx along the same path that is taken by the muscle precursors, and markers for the lateral mesoderm and overlying ectoderm precede those of other tissues (Lours-Calet et al. 2014). Lineage tracing experiments revealed that this extension of marker gene expression is due to cells moving along this circumpharyngeal path (Lours-Calet et al. 2014; Fig. 8). Specifically, the lateral mesoderm originating from the level of the first somite leads the procession, with the lateral mesoderm originating from the level of somite 2 following and embedding the hypopharyngeal muscle precursors; more caudal lateral mesoderm becomes displaced caudally. Studies on cells moving as a tissue sheet have shown that the moving sheet is able to drag non-migratory cells along (reviewed in (Montell 2008). Thus, it can be speculated that the newly discovered occipital cell movements, deep in the evolution of vertebrates, provided the original transport system for the initially non-migratory hypopharyngeal muscle cells.

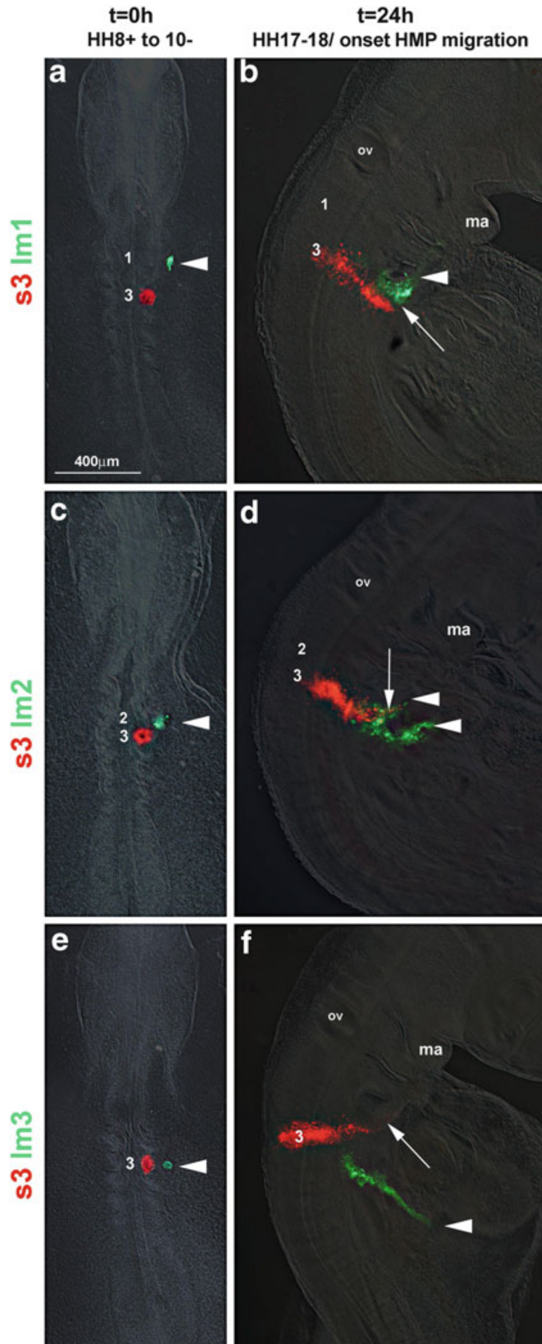


Fig. 8 An alternative mode of hypaxial cell transport at the head–neck interface. When genes required for the emigration of limb muscle precursors are mutated, limb muscles fail to form. Yet in most cases, hypobranchial muscle formation is merely delayed. This figure shows DiO-labelings (*green*) of the lateral mesoderm and DiI labelling (*red*) of a somite destined to produce

7 Outlook

Hypaxial muscles have been remodelled quite extensively during the evolution of vertebrates, and many aspects of their development have been deciphered. However, a number of questions, mainly surrounding migratory muscle precursors, remain: is it indeed possible that these cells evolved earlier than the emergence of osteichthyans, and is the underlying molecular programme an adaptation of programmes used for the dorsal and ventral fin muscles? And how does the formation of migratory muscle precursors for the fins/limbs relate to the release of individual cells to form the body wall muscle in frog (Martin and Harland 2001, 2006), the contribution of migratory cells to the rostral body wall in teleosts (Windner et al. 2011) or the de-epithelialisation of the somitic lips when the outgrowing somite reaches the ventral midline in amniotes (Christ et al. 1983)? Is it possible that, even though the cells destined to contribute to the body wall are mesenchymal, they migrate as sheet rather than individual cells? And what are the underlying molecular mechanisms? These questions may seem to have mainly academic merit, yet answers may provide knowledge and understanding for the therapy of birth defects such as gastroschisis or for the reconstruction or regeneration of muscle and limbs. Recently, it has been suggested that a mutation of the human *LBX1* gene may be responsible for the myopathy and severe vertebral column malformation in a patient (Fernandez-Jaen et al. 2014), reinforcing how basic research informs Medicine.

Acknowledgements We are grateful to Mohi U. Ahmed, Ashish K. Maurya, Louise Cheng, Erika C. Jorge, Pascal Maire, M. Albert Basson and Philip W. Ingham for agreeing to include currently unpublished data in this review article. The work was supported by the Human Frontier Science Program, Grant No R6Y0056/2004-C201, the European Network of Excellence Myores, Grant No EU LSHG-CT-2004-511978 MYORES, the Association Française contre les Myopathies, Grant No CL/NM/2005.2088/Following number 11378, Groupe A07/06/05 and CA 01/07/05, DRM05/BUIOCE/REFAP/MUSAI/ANGLE.

Fig. 8 (continued) hypobranchial muscle precursors (HMP) in a 36 hours chicken embryo (**a**,**c**,**e**). The embryos were analysed 24 h later when hypobranchial muscle precursors start to emigrate (**b**,**d**,**f**). Notably, all occipital lateral mesoderm moves ventrolaterally. However, eventually the streams of cells deviate. Cells originating from a position next to the most rostral somite (somite 1) take a rostral path along the floor of the pharynx which anticipates the course of the HMP (**a**,**b**). Lateral mesoderm from the level of somite 2 contributes both to the rostrally and ventrally—caudally directed stream. HMP become embedded in the rostrally projecting stream (**c,d**). Cells from the level of the third somite project exclusively caudally and are out of the way when HMP start to emigrate (**e**, **f**). This suggests extensive cells movements at the head–trunk interface. It furthermore suggests that there is a rostrally directed stream that is suited to carry non-migratory cells along. This stream is conserved and may represent the evolutionarily ancestral way of muscle precursor transport. *lm* lateral mesoderm, *ma* mandibular arch, *ov* otic vesicle, *s* somite

References

Ahmed MU, Cheng L, Dietrich S (2006) Establishment of the epaxial-hypaxial boundary in the avian myotome. Dev Dyn 235(7):1884–1894

Alvares LE, Schubert FR, Thorpe C, Mootoosamy RC, Cheng L, Parkyn G, Lumsden A, Dietrich S (2003) Intrinsic, Hox-dependent cues determine the fate of skeletal muscle precursors. Dev Cell 5(3):379–390

Beattie CE, Eisen JS (1997) Notochord alters the permissiveness of myotome for pathfinding by an identified motoneuron in embryonic zebrafish. Development 124(3):713–720

Ben-Yair R, Kalcheim C (2008) Notch and bone morphogenetic protein differentially act on dermomyotome cells to generate endothelium, smooth, and striated muscle. J Cell Biol 180 (3):607–618. doi:10.1083/jcb.200707206

Bladt F, Riethmacher D, Isenmann S, Aguzzi A, Birchmeier C (1995) Essential role for the c-met receptor in the migration of myogenic precursor cells into the limb bud. Nature 376 (6543):768–771. doi:10.1038/376768a0

Bober E, Franz T, Arnold H-H, Gruss P, Tremblay P (1994) Pax-3 is required for the development of limb muscles: a possible role for the migration of dermomyotomal muscle progenitor cells. Development 120:603–612

Borycki A-G, Li J, Jin F, Emerson CP (1999) Pax3 functions in cell survival and in Pax7 regulation. Development 126:1665–1674

Brohmann H, Jagla K, Birchmeier C (2000) The role of Lbx1 in migration of muscle precursor cells. Development 127(2):437–445

Brunet I, Di Nardo AA, Sonnier L, Beurdeley M, Prochiantz A (2007) The topological role of homeoproteins in the developing central nervous system. Trends Neurosci 30(6):260–267

Bryson-Richardson RJ, Currie PD (2008) The genetics of vertebrate myogenesis. Nat Rev Genet 9 (8):632–646

Buckingham M (2006) Myogenic progenitor cells and skeletal myogenesis in vertebrates. Curr Opin Genet Dev 16(5):525–532

Chen F, Liu KC, Epstein JA (1999) Lbx2, a novel murine homeobox gene related to the Drosophila ladybird genes is expressed in the developing urogenital system, eye and brain. Mech Dev 84 (1–2):181–184

Cheng L, Alvares LE, Ahmed MU, El-Hanfy AS, Dietrich S (2004) The epaxial-hypaxial subdivision of the avian somite. Dev Biol 274(2):348–369

Chevallier A, Kieny M, Mauger A (1977) Limb-somite relationship: origin of the limb musculature. J Embryol Exp Morphol 41:245–258

Christ B, Jacob HJ, Jacob M (1977) Experimental analysis of the origin of the wing musculature in avian embryos. Anat Embryol 150:171–186

Christ B, Jacob M, Jacob HJ (1983) On the origin and development of the ventrolateral abdominal muscles in the avian embryo. An experimental and ultrastructural study. Anat Embryol 166:87–101

Clack JA (2002) Gaining ground. The origin and evolution of tetrapods. Indiana University Press, Bloomington, IN

Cohn MJ, Izpisua-Belmonte JC, Abud H, Heath JK, Tickle C (1995) Fibroblast growth factors induce additional limb development from the flank of chick embryos. Cell 80(5):739–746

Cole NJ, Hall TE, Don EK, Berger S, Boisvert CA, Neyt C, Ericsson R, Joss J, Gurevich DB, Currie PD (2011) Development and evolution of the muscles of the pelvic fin. PLoS Biol 9 (10):1001168. doi:10.1371/journal.pbio.1001168

Currie PD, Ingham PW (1996) Induction of a specific muscle cell type by a hedgehog-like protein in zebrafish. Nature 382(6590):452–455

Devoto SH, Melancon E, Eisen JS, Westerfield M (1996) Identification of separate slow and fast muscle precursor cells in vivo, prior to somite formation. Development 122(11):3371–3380

Dietrich S (1999) Regulation of hypaxial muscle development. Cell Tissue Res 296(1):175–182
Dietrich S, Abou-Rebyeh F, Brohmann H, Bladt F, Sonnenberg-Riethmacher E, Yamaai T, Lumsden A, Brand-Saberi B, Birchmeier C (1999) The role of SF/HGF and c-Met in the development of skeletal muscle. Development 126(8):1621–1629
Dietrich S, Schubert FR, Healy C, Sharpe PT, Lumsden A (1998) Specification of the hypaxial musculature. Development 125(12):2235–2249
Durland JL, Sferlazzo M, Logan M, Burke AC (2008) Visualizing the lateral somitic frontier in the Prx1Cre transgenic mouse. J Anat 212(5):590–602. doi:10.1111/j.1469-7580.2008.00879.x
Fan CM, Lee CS, Tessier-Lavigne M (1997) A role for WNT proteins in induction of dermomyotome. Dev Biol 191(1):160–165
Fernandez-Jaen A, Suela J, Fernandez-Mayoralas DM, Fernandez-Perrone AL, Wotton KR, Dietrich S, Castellanos MD, Cigudosa JC, Calleja-Perez B, Lopez-Martin S (2014) Microduplication 10q24.31 in a Spanish girl with scoliosis and myopathy: the critical role of LBX. Am J Med Genet A 168:2074–8
Fetcho JR (1987) A review of the organization and evolution of motoneurons innervating the axial musculature of vertebrates. Brain Res 434(3):243–280
Franz T, Kothary R, Surani MAH, Halata Z, Grim M (1993) The Splotch mutation interferes with muscle development in the limbs. Anat Embryol 187:153–160
Freitas R, Gomez-Skarmeta JL, Rodrigues PN (2014) New frontiers in the evolution of fin development. J Exp Zool (Mol Dev Evol) 9999B:1–13
Freitas R, Zhang G, Cohn MJ (2006) Evidence that mechanisms of fin development evolved in the midline of early vertebrates. Nature 442(7106):1033–1037
Gans C, Northcutt RG (1983) Neural crest and the origin of vertebrates: a new head. Science 220:268–274
Goodrich ES (1958) Studies on the structure and development of vertebrates, 2nd edn. Dover, New York
Gray H (1995) Gray's anatomy, 38th edn. Churchill Livingstone, Edinburgh, London
Grifone R, Demignon J, Giordani J, Niro C, Souil E, Bertin F, Laclef C, Xu PX, Maire P (2007) Eya1 and Eya2 proteins are required for hypaxial somitic myogenesis in the mouse embryo. Dev Biol 302(2):602–616
Grifone R, Demignon J, Houbron C, Souil E, Niro C, Seller MJ, Hamard G, Maire P (2005) Six1 and Six4 homeoproteins are required for Pax3 and Mrf expression during myogenesis in the mouse embryo. Development 132(9):2235–2249
Gross MK, Moran-Rivard L, Velasquez T, Nakatsu MN, Jagla K, Goulding M (2000) Lbx1 is required for muscle precursor migration along a lateral pathway into the limb. Development 127(2):413–424
Hammond KL, Baxendale S, McCauley DW, Ingham PW, Whitfield TT (2009) Expression of patched, prdm1 and engrailed in the lamprey somite reveals conserved responses to Hedgehog signaling. Evol Dev 11(1):27–40
Hayashi K, Ozawa E (1995) Myogenic cell migration from somites is induced by tissue contact with medial region of the presumptive limb mesoderm in chick embryos. Development 121:661–669
Jagla K, Dollé P, Mattei M-G, Jagla T, Schuhbaur B, Dretzen G, Bellard F, Bellard M (1995) Mouse Lbx1 and human LBX1 define a novel mammalian homeobox gene family related to the Drosophila ladybird genes. Mech Dev 53:345–356
Jagla T, Bellard F, Lutz Y, Dretzen G, Bellard M, Jagla K (1998) ladybird determines cell fate decisions during diversification of Drosophila somatic muscles. Development 125(18):3699–3708
Kanamoto T, Terada K, Yoshikawa H, Furukawa T (2006) Cloning and expression pattern of lbx3, a novel chick homeobox gene. Gene Expr Patterns 6(3):241–246
Kardon G, Harfe BD, Tabin CJ (2003) A Tcf4-positive mesodermal population provides a prepattern for vertebrate limb muscle patterning. Dev Cell 5(6):937–944

Kiehn O (2011) Development and functional organization of spinal locomotor circuits. Curr Opin Neurobiol 21(1):100–109. doi:10.1016/j.conb.2010.09.004

Kusakabe R, Kuraku S, Kuratani S (2011) Expression and interaction of muscle-related genes in the lamprey imply the evolutionary scenario for vertebrate skeletal muscle, in association with the acquisition of the neck and fins. Dev Biol 350(1):217–227. doi:10.1016/j.ydbio.2010.10.029

Layalle S, Volovitch M, Mugat B, Bonneaud N, Parmentier ML, Prochiantz A, Joliot A, Maschat F (2011) Engrailed homeoprotein acts as a signaling molecule in the developing fly. Development 138(11):2315–2323. doi:10.1242/dev.057059

Lewis KE, Eisen JS (2003) From cells to circuits: development of the zebrafish spinal cord. Prog Neurobiol 69(6):419–449

Lours-Calet C, Alvares LE, El-Hanfy AS, Gandesha S, Walters EH, Sobreira DR, Wotton KR, Jorge EC, Lawson JA, Kelsey Lewis A, Tada M, Sharpe C, Kardon G, Dietrich S (2014) Evolutionarily conserved morphogenetic movements at the vertebrate head-trunk interface coordinate the transport and assembly of hypopharyngeal structures. Dev Biol 390(2):231–246. doi:10.1016/j.ydbio.2014.03.003

Mackenzie S, Walsh FS, Graham A (1998) Migration of hypoglossal myoblast precursors. Dev Dyn 213(4):349–358

Martin BL, Harland RM (2001) Hypaxial muscle migration during primary myogenesis in Xenopus laevis. Dev Biol 239(2):270–280. doi:10.1006/dbio.2001.0434

Martin BL, Harland RM (2006) A novel role for lbx1 in Xenopus hypaxial myogenesis. Development 133(2):195–208

Matsuura M, Nishihara H, Onimaru K, Kokubo N, Kuraku S, Kusakabe R, Okada N, Kuratani S, Tanaka M (2008) Identification of four Engrailed genes in the Japanese lamprey, Lethenteron japonicum. Dev Dyn 237(6):1581–1589

Maurya AK, Tan H, Souren M, Wang X, Wittbrodt J, Ingham PW (2011) Integration of Hedgehog and BMP signalling by the engrailed2a gene in the zebrafish myotome. Development 138 (4):755–765. doi:10.1242/dev.062521

Mennerich D, Braun T (2001) Activation of myogenesis by the homeobox gene Lbx1 requires cell proliferation. EMBO J 20(24):7174–7183

Mennerich D, Schafer K, Braun T (1998) Pax-3 is necessary but not sufficient for lbx1 expression in myogenic precursor cells of the limb. Mech Dev 73(2):147–158

Minchin JE, Williams VC, Hinits Y, Low S, Tandon P, Fan CM, Rawls JF, Hughes SM (2013) Oesophageal and sternohyal muscle fibres are novel Pax3-dependent migratory somite derivatives essential for ingestion. Development 140(14):2972–2984. doi:10.1242/dev.090050

Montell DJ (2008) Morphogenetic cell movements: diversity from modular mechanical properties. Science 322(5907):1502–1505

Murakami Y, Tanaka M (2011) Evolution of motor innervation to vertebrate fins and limbs. Dev Biol 355(1):164–172. doi:10.1016/j.ydbio.2011.04.009

Murphy MM, Lawson JA, Mathew SJ, Hutcheson DA, Kardon G (2011) Satellite cells, connective tissue fibroblasts and their interactions are crucial for muscle regeneration. Development 138 (17):3625–3637. doi:10.1242/dev.064162

Neyt C, Jagla K, Thisse C, Thisse B, Haines L, Currie PD (2000) Evolutionary origins of vertebrate appendicular muscle. Nature 408(6808):82–86

Nowicki JL, Burke AC (2000) Hox genes and morphological identity: axial versus lateral patterning in the vertebrate mesoderm. Development 127(19):4265–4275

Ochi H, Westerfield M (2009) Lbx2 regulates formation of myofibrils. BMC Dev Biol 9:13. doi:10.1186/1471-213X-9-13

Odemis V, Lamp E, Pezeshki G, Moepps B, Schilling K, Gierschik P, Littman DR, Engele J (2005) Mice deficient in the chemokine receptor CXCR4 exhibit impaired limb innervation and myogenesis. Mol Cell Neurosci 30(4):494–505

Onimaru K, Shoguchi E, Kuratani S, Tanaka M (2011) Development and evolution of the lateral plate mesoderm: comparative analysis of amphioxus and lamprey with implications for the acquisition of paired fins. Dev Biol 359(1):124–136. doi:10.1016/j.ydbio.2011.08.003

Pourquié O, Fan CM, Coltey M, Hirsinger E, Watanabe Y, Bréant C, Francis-West P, Brickell P, Tessier-Lavigne M, Le Douarin NM (1996) Lateral and axial signals involved in avian somite patterning: a role for BMP4. Cell 84(3):461–471

Prunotto C, Crepaldi T, Forni PE, Ieraci A, Kelly RG, Tajbakhsh S, Buckingham M, Ponzetto C (2004) Analysis of Mlc-lacZ Met mutants highlights the essential function of Met for migratory precursors of hypaxial muscles and reveals a role for Met in the development of hyoid arch-derived facial muscles. Dev Dyn 231(3):582–591

Sambasivan R, Kuratani S, Tajbakhsh S (2011) An eye on the head: the development and evolution of craniofacial muscles. Development 138(12):2401–2415. doi:10.1242/dev.040972

Scaal M, Bonafede A, Dathe V, Sachs M, Cann G, Christ B, Brand-Saberi B (1999) SF/HGF is a mediator between limb patterning and muscle development. Development 126(21):4885–4893

Schäfer K, Braun T (1999) Early specification of limb muscle precursor cells by the homeobox gene Lbx1h. Nat Genet 23(2):213–216

Shearman RM, Burke AC (2009) The lateral somitic frontier in ontogeny and phylogeny. J Exp Zool B Mol Dev Evol 312(6):603–612. doi:10.1002/jez.b.21246

Tajbakhsh S, Borello U, Vivarelli E, Kelly R, Papkoff J, Duprez D, Buckingham M, Cossu G (1998) Differential activation of Myf5 and MyoD by different Wnts in explants of mouse paraxial mesoderm and the later activation of myogenesis in the absence of Myf5. Development 125(21):4155–4162

Thisse B, Heyer V, Lux A, Alunni V, Degrave A, Seiliez I, Kirchner J, Parkhill JP, Thisse C (2004) Spatial and temporal expression of the zebrafish genome by large-scale in situ hybridization screening. Methods Cell Biol 77:505–519

Tosney KW (1987) Proximal tissues and patterned neurite outgrowth at the lumbosacral level of the chick embryo: deletion of the dermamyotome. Dev Biol 122(2):540–558

Tosney KW, Landmesser LT (1985) Growth cone morphology and trajectory in the lumbosacral region of the chick embryo. J Neurosci 5(9):2345–2358

Trainor P, Krumlauf R (2000) Plasticity in mouse neural crest cells reveals a new patterning role for cranial mesoderm. Nat Cell Biol 2(2):96–102

Tremblay P, Dietrich S, Meriskay M, Schubert FR, Li Z, Paulin D (1998) A crucial role for *Pax3* in the development of the hypaxial musculature and the long-range migration of muscle precursors. Dev Biol 203:49–61

Tsuchida T, Ensini M, Morton SB, Baldassare M, Edlund T, Jessell TM, Pfaff SL (1994) Topographic organization of embryonic motor neurons defined by expression of LIM homeobox genes. Cell 79(6):957–970

Tulenko FJ, McCauley DW, Mackenzie EL, Mazan S, Kuratani S, Sugahara F, Kusakabe R, Burke AC (2013) Body wall development in lamprey and a new perspective on the origin of vertebrate paired fins. Proc Natl Acad Sci U S A 110(29):11899–11904. doi:10.1073/pnas.1304210110

Turney BW, Rowan-Hull AM, Brown JM (2003) The innervation of FGF-induced additional limbs in the chick embryo. J Anat 202(1):83–92

Vasyutina E, Stebler J, Brand-Saberi B, Schulz S, Raz E, Birchmeier C (2005) CXCR4 and Gab1 cooperate to control the development of migrating muscle progenitor cells. Genes Dev 19 (18):2187–2198

Watanabe S, Kondo S, Hayasaka M, Hanaoka K (2007) Functional analysis of homeodomain-containing transcription factor Lbx1 in satellite cells of mouse skeletal muscle. J Cell Sci 120 (Pt 23):4178–4187. doi:10.1242/jcs.011668

Windner SE, Steinbacher P, Obermayer A, Kasiba B, Zweimueller-Mayer J, Stoiber W (2011) Distinct modes of vertebrate hypaxial muscle formation contribute to the teleost body wall musculature. Dev Genes Evol 221(3):167–178. doi:10.1007/s00427-011-0369-1

Wotton KR, Weierud FK, Dietrich S, Lewis KE (2008) Comparative genomics of Lbx loci reveals conservation of identical Lbx ohnologs in bony vertebrates. BMC Evol Biol 8:171–186

Yang X-M, Vogan K, Gros P, Park M (1996) Expression of the met receptor tyrosine kinase in muscle progenitor cells in somites and limbs is absent in Splotch mice. Development 122 (7):2163–2171

Zhu M, Yu X, Ahlberg PE, Choo B, Lu J, Qiao T, Qu Q, Zhao W, Jia L, Blom H, Zhu Y (2013) A Silurian placoderm with osteichthyan-like marginal jaw bones. Nature 502(7470):188–193. doi:10.1038/nature12617

Skeletal Myogenesis in the Zebrafish and Its Implications for Muscle Disease Modelling

David Gurevich, Ashley Siegel, and Peter D. Currie

Abstract Current evidence indicates that post-embryonic muscle growth and regeneration in amniotes is mediated almost entirely by stem cells derived from muscle progenitor cells (MPCs), known as satellite cells. Exhaustion and impairment of satellite cell activity is involved in the severe muscle loss associated with degenerative muscle diseases such as Muscular Dystrophies and is the main cause of age-associated muscle wasting. Understanding the molecular and cellular basis of satellite cell function in muscle generation and regeneration (myogenesis) is critical to the broader goal of developing treatments that may ameliorate such conditions.

Considerable knowledge exists regarding the embryonic stages of amniote myogenesis. Much less is known about how post-embryonic amniote myogenesis proceeds, how adult myogenesis relates to embryonic myogenesis on a cellular or genetic level. Of the studies focusing on post-embryonic amniote myogenesis, most are post-mortem and in vitro analyses, precluding the understanding of cellular behaviours and genetic mechanisms in an undisturbed in vivo setting. Zebrafish are optically clear throughout much of their post-embryonic development, facilitating their use in live imaging of cellular processes. Zebrafish also possess a compartment of MPCs, which appear similar to satellite cells and persist throughout the post-embryonic development of the fish, permitting their use in examining the contribution of these cells to muscle tissue growth and regeneration.

D. Gurevich • A. Siegel
Australian Regenerative Medicine Institute, Monash University, Level 1, Building 75, Wellington Road, Clayton, VIC 3800, Australia

P.D. Currie (✉)
Australian Regenerative Medicine Institute, Monash University, Level 1, Building 75, Wellington Road, Clayton, VIC 3800, Australia

EMBL Australia, Melbourne Node, Monash University, Level 1, Building 75, Wellington Road, Clayton, VIC 3800, Australia
e-mail: peter.currie@emblaustralia.org; peter.currie@monash.edu

© Springer-Verlag Berlin Heidelberg 2015

B. Brand-Saberi (ed.), *Vertebrate Myogenesis*, Results and Problems in Cell Differentiation 56, DOI 10.1007/978-3-662-44608-9_3

1 Introduction

Myogenesis is a key process in the development and survival of almost all animals, with many of the physiological processes and molecular mechanisms involved in myogenesis being conserved throughout vertebrates and invertebrates (Figeac et al. 2007). Understanding the mechanisms of myogenesis is of particular interest due to the expense incurred by both individuals and society when this process is compromised—muscular dystrophies such as Duchenne Muscular Dystrophy (DMD) affects one in every 3500 males with varying severity of muscle weakness and premature mortality (Fairclough et al. 2011), while the cost associated with the age-related muscle wasting of sarcopenia is estimated at $20 billion dollars in the USA alone (Frontera et al. 2012). The study of myogenesis has therefore attracted an abundance of attention from investigators, particularly studies into the initial embryonic myogenic phases of amniotes that give rise to the primary myotome and the precursors of adult musculature (Bryson-Richardson and Currie 2008). Using various genetic and cellular manipulations, investigators have unravelled the main transcriptional controls and cell interactions involved in primary myogenesis. The considerable amount of knowledge regarding primary myogenesis is due to the relative ease of examining this process—it happens early during development, and embryos are simpler to generate, handle, and manipulate than organisms at later stages. Much less is known about how later stages of myogenesis proceed, and how the processes of post-embryonic and embryonic myogenesis compare on a cellular or genetic level. Of studies focusing on post-embryonic myogenesis, most have been limited to post-mortem analysis of genetic knock-outs and in vitro analysis on cultured myogenic stem cells and single fibres (Cornelison 2008). Such studies invariably disturb the normal environmental niche occupied by the stem cells of interest, affecting the behaviour of these cells and consequently the relevance of conclusions drawn from these investigations with regard to true in vivo outcomes (Cornelison 2008). Furthermore, these studies provide no opportunity to examine the behaviour of muscle stem cells in real time in living tissue. Similar limitations affect studies on muscle disease models in amniotes. While there are numerous models of muscle disease, such as DMD models in mice and dogs, the assays used both to identify the baseline characteristics of the disease and to ascertain whether applied putative therapeutic strategies have provided an amelioration of the symptoms are generally limited to muscle sections taken from euthanized animals (Guyon et al. 2007).

Compared to traditional models such as mice, rats, or chick, zebrafish possess numerous characteristics that facilitate their use as a model organism for vertebrate muscle growth, repair, and disease. They develop rapidly external to the mother and are optically clear throughout much of their post-embryonic development, permitting live imaging of cellular and biochemical events involved in growth and repair processes of post-embryonic tissues. Furthermore, the recent generation of transparent zebrafish such as the *casper* mutant extends the scope of possible imaging experiments that can be performed (White et al. 2008). In particular, zebrafish

skeletal muscle shares many molecular and structural similarities to human muscle and is one of the largest and most discernible organ systems in fish (Gibbs et al. 2013). Coupled with a high genetic tractability, these qualities allow the use of zebrafish in generating tissue-specific transgenic lines and subsequently live imaging of labelled tissues and cells (Goldsmith and Jobin 2012). Zebrafish are also capable of generating large numbers of offspring and absorb many drugs simply by being immersed in them, thus promoting their use in high-throughput drug screening (Helenius and Yeh 2012). Establishing how the zebrafish generates post-embryonic musculature, what cellular events are involved, and how these compare to the amniote are therefore of key importance to furthering its use as a model for muscle development and regeneration.

This chapter provides a comparison of embryonic skeletal muscle establishment in the amniote and the zebrafish, focusing specifically on trunk musculature. Post-embryonic skeletal muscle development and regeneration post-injury for both amniotes and teleosts is also covered, focusing in particular on muscle stem cell location, behaviour, and heterogeneity. Finally, the implications for modelling human diseases using the knowledge so far acquired via the zebrafish are explored in further detail.

2 Developmental Muscle Formation

The first phase in myogenesis involves the establishment of the myotome from embryonic structures known as somites. This myotome then expands during fetal and early postnatal development, transitioning into the more integrated and complex adult musculature. Finally, this adult musculature needs to be scaled up appropriately as the organism approaches its adult size in the first instance, and must subsequently be maintained throughout adulthood by regeneration and repair in instances of injury or disease. These three broadly defined phases produce muscles that possess some genetic and morphological differences, while ultimately having a single aim—to provide the required movement capacity once fully established (Murphy and Kardon 2011). Due to differentiated muscle tissue being both post-mitotic and syncitial, it is generated by mononucleate myogenic progenitor cells capable of proliferating, differentiating and fusing to one another and to existing muscle fibres. The following section will compare the zebrafish to amniotes in terms of cellular movements and genetic controls that eventually lead to the establishment of the early myotome and the myogenic progenitors, focusing in particular on functional similarities and differences.

2.1 Comparisons in Somitogenesis: Dermomyotome Versus External Cell Layer

In amniotes, all skeletal muscle bar craniofacial musculature is derived from somites—transient spherical structures that originate from the paraxial mesoderm along either side of the neural tube (Tajbakhsh and Buckingham 2000; Braun and Gautel 2011). Somitogenesis begins at embryonic day 8–8.5 (E8–8.5), or at mid-gestation, in the mouse (Downs and Davies 1993), and at E1, or at early incubation, in the chick (Hamburger and Hamilton 1951). By comparison, somitogenesis in zebrafish begins at approximately 10.5 h post-fertilization, a relatively early point in an embryonic development that lasts for approximately 72 h (Kimmel et al. 1995). The specific genes involved and pattern of expression utilized are largely conserved between teleosts and amniotes, as previously reviewed (Holley 2007).

Somites subsequently develop distinct polarity and compartments. In amniotes, the ventral aspect of somites undergoes an epithelial-to-mesenchymal transition (EMT), which drives the formation of the sclerotome, a somitic region responsible for giving rise to cartilage, bone, and connective tissue (Brand-Saberi and Christ 2000; Kalcheim and Ben-Yair 2005). The dorsal somitic aspect maintains its epithelial nature and gives rise to a structure known as the dermomyotome, which is the source of the dermis and skeletal musculature of the trunk and limbs (Ben-Yair and Kalcheim 2005; Cossu et al. 2000). The muscle precursors from the dermomyotome express the paired box transcription factors *Pax3* and *Pax7*, as well as low levels of the basic helix-loop-helix transcription factor *Myf5* (Jostes et al. 1990; Kiefer and Hauschka 2001; Goulding et al. 1991). These, along with the early myogenic marker *Myod* and late myogenic markers *Myogenin* and *Myf6 (Mrf4)*, are all important players in the transcriptional regulatory cascade governing subsequent muscle specification and differentiation that comprises primary myogenesis.

By contrast, the establishment of somite polarity in zebrafish leads to a different manner of morphological segmentation, whereby boundary cells between somites at the most rostral portion of the embryo undergo mesenchymal-to-epithelial transitions, forming an epithelial sheet around loosely organized mesenchymal cells (Stickney et al. 2000). Along with this, specification and differentiation events transpire relatively earlier in the zebrafish. Genetic studies have shown that precursors to trunk somites are specified before gastrulation (Szeto and Kimelman 2006). Myogenic commitment also occurs much faster in the fish, with the first expression of *myod* occurring in notochord-adjacent adaxial cells towards the end of gastrulation (Weinberg 1996), as opposed to the expression of myogenic genes in amniote pioneer cells (Venters et al. 1999). Expression of myogenic genes in somitic compartments is also observed at this stage. Meanwhile, *pax3* and *pax7* expression is restricted to the anterior somitic compartment, indicating the role that these cells play in the establishment of myogenic precursor cells (Hollway et al. 2007).

Importantly, these early myogenic events and expression of these myogenic factors in the zebrafish happen without the establishment of a morphological equivalent of the dermomyotome, a striking difference to the normal process in amniotes. Instead, once early gene expression and somite polarity is established, a whole somite rotation event occurs whereby the anterior portion of the somite migrates to the lateral most aspect of the myotome (Hollway et al. 2007). This anterior somitic compartment gives rise to a number of different cell types, including populations of muscle progenitors that express *pax3* and *pax7*, localize to the lateral surface of the myotome as a thin layer of cells termed the external cell layer (ECL), and appear to serve a similar role to the amniote dermomyotome by giving rise to myogenic progenitors and myoblasts (Hollway et al. 2007; Devoto et al. 2006; Bryson-Richardson and Currie 2008; Stellabotte et al. 2007; Waterman 1969).

Following segmentation, the zebrafish somite is therefore divided into myotome, sclerotome, and the aforementioned functional equivalent to the dermomyotome (the ECL), which respectively give rise to the musculature, skeletal elements and myogenic progenitors responsible for subsequent growth of skeletal muscle (Stickney et al. 2000; Devoto et al. 2006; Morin-Kensicki and Eisen 1997). This shows an overlap between somitogenesis and primary myogenesis in the fish that does not exist in the more regimented process of myogenesis in the amniote. A possible explanation for this observation is the obvious advantage to more quickly acquiring the ability to move for externally fertilized animals such as zebrafish, primarily in order to avoid predation. Indeed, when prematurely hatched from their protective chorion, zebrafish are capable of generating an escape response to physical stimulus by 27 h post-fertilization, or roughly one-third of the way through their embryonic development (Saint-Amant and Drapeau 1998). Therefore, the ability of the zebrafish to generate coordinated movement occurs relatively much earlier than in the amniote, with chickens only displaying directed and coordinated bouts of movement in the last few days of their 21-day incubation period (Oppenheim 1974). Further examination has indicated that amniote myotomal muscle undergoes innervation at a much later relative timepoint in development compared to fish and amphibians, thereby providing a potential mechanism for the observed delay in the onset of primary myogenesis (Deries et al. 2008).

2.2 Primary and Secondary Myogenesis

Establishment of skeletal muscle in amniotes involves an initial differentiation event where post-mitotic myocytes, expressing the myogenic specification markers *Myf5* and *Myod*, migrate under the developing dermomyotome and align themselves along the axis of the embryo (Gros et al. 2004; Pownall et al. 2002). These myocytes form “pioneer” fibres and act as a scaffold for the developing myotome (Kahane et al. 2002; Denetclaw and Ordahl 2000). Subsequently, post-mitotic myocytes delaminate from the four lips of the dermomyotome as waves of cells,

migrate inwards and form the primary myotubes that make up the definitive trunk musculature. The central block of the dermomyotome that undergoes EMT last is comprised of cells that are highly proliferative, fusing to each other or to existing primary fibres and thereby resulting in the dramatic growth and transformation involved in the primary myotome transitioning from simple, metameric segmented myotome to complex, definitive trunk musculature (Cinnamon et al. 1999; Deries et al. 2010). This central block also gives rise to the satellite cells, myogenic cells that withdraw from the cell cycle, maintain *Pax3* and *Pax7* expression while moving into direct proximity to established muscle fibres and, upon activation, are responsible for generating the myoblasts involved in postnatal muscle growth and repair (Relaix et al. 2005; Gros et al. 2005; Ben-Yair and Kalcheim 2005; Lepper and Fan 2010). Each of these types of myogenic fibres and cells (pioneer, primary, secondary, satellite) is formed via processes distinct from one another, with particular genetic controls (Murphy and Kardon 2011). The resultant amniote skeletal muscle is composed of a mixture of muscle fibre types possessing differing characteristics, such as expression of a range of myosin heavy chain isoforms leading to a spectrum of fibres—from slow-twitch fibres capable of repeated submaximal contractions, to fast-twitch fibres capable of limited maximal contractions. This muscle composition possesses a degree of plasticity, being able to change in response to various stimuli, such as hormones and neural input, which in turn can be influenced by exogenous variables such as nutrition, imposed workload, and age (Schiaffino and Reggiani 2011; Davis and Fiorotto 2009).

In contrast to amniotes, zebrafish myogenic precursor cells that are part of the presomitic mesoderm begin to localize medially against the notochord, and are known as the adaxial cells (Devoto et al. 1996). The commitment of adaxial cells via promotion of MRF expression depends on Sonic Hedghog (shh) signalling from the notochord (Blagden et al. 1997; Currie and Ingham 1996; Coutelle et al. 2001; Weinberg et al. 1996), a morphogen that plays a similar role in the amniote (Pirskanen et al. 2000). These adaxial myoblasts express both fast-twitch and slow-twitch myosin heavy chain (*myhc)*, again a phenotype shared with primary fibres of the amniote myotome (Bryson-Richardson et al. 2005; Kalcheim et al. 1999; Kahane et al. 1998). A subset of these cells are the first to differentiate and become the muscle pioneer population, which remain adjacent to the notochord at the midline of the embryo and potentially act as scaffolding for subsequent muscle fibres, much like the pioneer fibres in the amniote (Felsenfeld et al. 1991; Waterman 1969). These first centrally mononucleate myocytes span the entire somite in a rostrocaudal direction (Blagden et al. 1997), again similar to the pattern seen in amniotes (Venters et al. 1999). Directly lateral to the muscle pioneer fibres, a specialized structure comprised of connective tissue sheets is formed. This structure is called the horizontal myosepta and together with the vertical myosepta separate the myotome into dorsal/ventral compartments and adjacent myomeres, respectively (Bone 1989; Currie and Ingham 2001). These areas of connective tissue are thought to function as a pathway for myogenic cell migration in the teleost (Stoiber 1996).

The rest of the adaxial population migrate from their position near the notochord to become a monolayer of mononucleate, elongated, and striated cells located subcutaneously on the lateral aspect of the myotome (Devoto et al. 1996; Blagden et al. 1997; Wolff et al. 2003). These are the aerobic, slow-twitch muscle fibres, marked by their expression of slow myosin heavy chain (*smyhc*) (Blagden et al. 1997). The segregation of this single population of adaxial cells into progenitors of both the muscle pioneer cells and superficial slow muscle fibres is performed via independent signalling of Sonic Hedgehog, Fibroblast Growth Factor (fgf) and Bone Morphogenetic Protein (bmp) pathways, which synergistically provides three dimensional coordination and control of adaxial cell fate (Dolez et al. 2011, Maurya et al. 2011; Nguyen-Chi et al. 2012). The second phase in zebrafish embryonic myogenesis occurs post somite rotation, involving the differentiation of the now lateral somitic compartment. After being passed by the migrating front of slow fibre precursors, these myoblasts undergo differentiation, expressing MRFs such as *myod* and *myogenin*, as well as fast glycolytic fibre markers such as fast myosin heavy chain (Devoto et al. 1996; Groves et al. 2005; Henry and Amacher 2004). Functionally, these cells may be considered to be the equivalent of the primary myotubes of amniote definitive trunk musculature. The generation of these lateral fast fibres depends on fgf8 signalling induced by retinoic acid in the somites and the anterior PSM, which in turn induces the aforementioned expression of myogenic factors (Hamade et al. 2006; Groves et al. 2005). By contrast, specification of the more medial fast fibres is fgf8 independent (Groves et al. 2005). Fgfs are also expressed by anterior somitic cells that will become the muscle progenitor cells of the ECL, potentially regulating their subsequent differentiation (Groves et al. 2005; Hammond et al. 2007). The ECL persists beyond embryonic myogenesis and continues to be a source of proliferative myogenic progenitors and myoblasts that contribute to further phases of skeletal muscle generation (Hollway et al. 2007). Work in the closely related pearlfish, - has shown maintenance of pax7-expressing cells at the position of the ECL throughout development, up to adulthood (Marschallinger et al. 2009; Steinbacher et al. 2011). This population of myogenic cells therefore appears to mirror the function of fetal myocytes, and later satellite cells, as has been recently reviewed (Siegel et al. 2013).

The above information shows both conservation and divergence of mechanisms underlying embryonic myogenesis when comparing zebrafish to amniotes. Functionally, there are quite clear parallels that could be drawn between zebrafish and amniote muscle establishment; the adaxial cells being equivalent to the pioneer fibres; the early fast fibres equivalent to the primary myotubes of the amniote; the intercalating fibres and cells from the ECL as the equivalents to, respectively, secondary myotube formation during fetal myogenesis and satellite cell establishment during postnatal myogenesis. Some of the main morphogens involved in amniote somitogenesis and early myogenesis—particularly Fgfs and Shh—play very similar roles in directing myogenesis, and at very similar relative timepoints. Myogenic genes that are important in amniote myogenesis are represented in the zebrafish embryonic myotome, with many playing the same specification or

differentiation role. However, myogenic commitment occurs relatively much earlier for some muscle tissue, such as the muscle pioneer cells, in the zebrafish compared to the amniote. This commitment and the events that follow segregate the zebrafish myotome into lateral slow muscle and medial fast muscle populations, again a very different arrangement to the amniote "salt-and-pepper" pattern of multiple muscle fibre types located in the same muscle. Finally, all of these myogenic events occur without a classical epithelial dermomyotome, although the teleost does appear to have a population that is functionally similar, the ECL.

3 Growth and Patterning in the Zebrafish

3.1 Establishing the Existence of Satellite Cell Equivalents in the Zebrafish

A number of transcription factors play a crucial role in embryonic, fetal, and adult myogenesis. The similarities between activation of satellite cells and somitic myogenesis suggest that post-embryonic myogenesis recapitulates elements of embryonic development. Therefore, further discussion of the mechanisms underlying myogenesis, particularly in the establishment of satellite cell equivalents in the zebrafish, requires an understanding of the specific roles played by these genetic markers and control elements.

The cellular migration marker *cMet* labels all satellite cells associated with myofibres from explanted murine skeletal muscle (Andermarcher et al. 1996; Cornelison and Wold 1997). *Pax7* is also expressed in murine mononucleate cells that either differentiate into myotubes or give rise to the satellite cell compartment (Horst et al. 2006). By contrast, lineage tracing studies in both chick and mouse limbs have shown *Pax3* cells to give rise to all embryonic, fetal and adult myoblasts, including all cells of the *Pax7* lineage (Schienda et al. 2006). Conditional ablation of these populations during early development results in no embryonic or fetal myogenesis (Hutcheson et al. 2009). Both Pax3 and Pax7 directly bind regulatory elements of Myf5 and MyoD, thereby regulating the expression of the myogenic program in chick and mice (Bajard et al. 2006; Hu et al. 2008). The basic helix-loop-helix genes *Myf5*, *MyoD*, *Myogenin*, and *Mrf4* are highly conserved myogenic regulatory factors (MRFs) expressed early in the murine skeletal muscle lineage (Rudnicki and Jaenisch 1995; Arnold and Braun 1996). Transforming cell types such as fibroblasts with any of these factors results in induction of myoblast traits in non-muscle cells, such as the ability to fuse into myotubes (Davis et al. 1987; Braun et al. 1990; Miner and Wold 1990; Edmondson and Olson 1989). *Myf5*, *MyoD*, and *Mrf4* are all expressed in myoblasts, while *MyoD*, *Mrf4*, and *Myogenin* are all expressed in the nuclei of differentiated myofibres (Ott et al. 1991; Bober et al. 1991; Sassoon et al. 1989; Voytik et al. 1993; Hinterberger

et al. 1991). Studies on loss-of-function mutants for various members of this family have demonstrated redundancy between certain family members in early myogenic specification by Myf5 and MyoD (Rudnicki et al. 1993; Kassar-Duchossoy et al. 2004), as well as important roles in later myogenic differentiation for Myogenin and Mrf4 (Hasty et al. 1993; Nabeshima et al. 1993; Valdez et al. 2000; Venuti et al. 1995; Rawls et al. 1998).

These same molecular markers and regulators are expressed during zebrafish myogenesis and appear to serve very similar functions. Once the early events of myogenesis are completed, *pax3* and *pax7* expression is seen in a single layer of dermomyotome-like flattened cells located externally to the myotome, the ECL. Both genes are highly conserved and, similar to the amniote, *pax3* is expressed in the somite prior to *pax7* (Seo et al. 1998; Hammond et al. 2007). Lineage analysis has shown these cells to contribute to embryonic and larval muscle (Hollway et al. 2007; Stellabotte et al. 2007). Inhibition of downstream myogenic signals such as *myf5* and *myod* results in an accumulation of undifferentiated *pax3/7*-expressing cells on the lateral surface of the somite (Hammond et al. 2007). Mononucleate cells expressing *pax7* appear to become associated with existing fibres at 72 h post-fertilization, and the use of transgenic lines has allowed the visualization of these cells contributing to expansion of the myotome, as well as assuming positions deeper within the myotome (Seger et al. 2011; Hollway et al. 2007). Some of these cells located deeper in the myotome express several markers specific to mammalian satellite cells, such as *cmet* (Hollway et al. 2007). These *pax7*- and *cmet*-expressing cells appear to be maintained at least during larval muscle development (Hollway et al. 2007; Marschallinger et al. 2009).

With regards to other elements of the transcriptional circuitry governing muscle cell specification and differentiation, all members of the MRF family have been identified in fish, showing high levels of sequence conservation compared to the amniote, with *myod* being most highly conserved (Hinits et al. 2007; Kobiyama et al. 1998; Rescan and Gauvry 1996). The first MRFs to be expressed in PSM, tailbud and early somites are *myf5* and *myod*, with *myf5* expression persisting in some fast muscle precursors (Coutelle et al. 2001; Stellabotte et al. 2007). Expression of *myf5* in newly generated fibres throughout larval development has also been detected (Seger et al. 2011). The loss-of-function *myf5* mutant shows no overt phenotype, but fails to thrive and dies during larval growth for reasons not yet elucidated (Hinits et al. 2009). By comparison, the investigated loss-of-function *myod* mutant results in a severe and lethal muscle phenotype, with delays in somite myogenesis and reduction in somite size and overall musculature (Hinits et al. 2011). *myod* function is also necessary for the formation of the lateral fast fibres, derived from the anterior somite (Hinits et al. 2009). Knockdown of either *myf5* or *myod* results in a delay of both slow and fast myogenesis (Schnapp et al. 2009; Hammond et al. 2007). When the function of both genes is compromised, most slow and fast muscle fails to form, and expression of *myogenin* and *mrf4* is absent in these double morphants (Hinits et al. 2009; Schnapp et al. 2009).

By contrast, loss-of-function *mrf4* and *myogenin* mutants are viable (Hinits et al. 2009). While not having a visible phenotype of its own, combining the *myogenin* and *myod* mutants exacerbates the phenotype seen in the *myod* mutant alone (Hinits et al. 2011). Newly differentiated muscle fibres from both slow and fast lineages express both of these genes (Hinits et al. 2007; Weinberg 1996). Knockdown of *mrf4* leads to defects in fibre organization, whereas forced early expression of *mrf4* leads to a partial rescue of double *myf5*/*myod* morphants (Wang et al. 2008; Schnapp et al. 2009). Expression of *mrf4* does not occur early enough to mark the early satellite cell equivalent precursor population, and only some fast muscle precursors are marked by *myogenin* (Schnapp et al. 2009; Hinits et al. 2007; Devoto et al. 2006).

Taken together, the information presented in this section describes a mechanism of generating satellite cell equivalents in the zebrafish that shares similarities to those present in amniote with respect to gene expression and resultant cell role. Furthermore, it appears that the MRFs in fish have similar functions to amniote MRFs in early muscle development. Myogenic commitment is still governed by *myf5*, *myod* and to a lesser extent *mrf4*, while terminal differentiation is still controlled mainly by *myog* and *mrf4*, with *myod* providing some redundancy. However, the exact input of these *pax7* and *cmet* satellite-like myogenic cells into later post-embryonic muscle development is still being elucidated, as is the heterogeneity of the resultant myogenic progenitors and myoblasts. These cells also remain in an interstitial position relative to muscle fibres for much longer in development than amniote satellite cells, which quickly adopt a sublaminal position (Hollway et al. 2007; Seger et al. 2011). This is perhaps due to the lack of basement lamina in the fish, a structure that only appears late in the development of the fish musculature (Hollway et al. 2007). What affect this difference in niche has on the zebrafish myogenic progenitors requires further investigation.

3.2 Growth Mechanisms: Comparison Between Amniote and Teleost

In the amniote, the generation of adult muscle via postnatal expansion of the myotome can be achieved by increasing the number of myofibres in the myotome (hyperplasia), increasing the size of existing myofibres (hypertrophy), or a combination of the two. Both of these processes are employed in the rat postnatally (Enesco and Puddy 1964; Ross et al. 1987), however mice, sheep, cattle, and humans appear to utilize hypertrophy in instances of normal juvenile growth, having reached their full complement of myofibres by birth (Ontell et al. 1984; Du et al. 2010; Stickland 1981). Hypertrophy via nuclear addition occurs up to the end of neonatal muscle growth and the beginning of true adult muscle growth (21 days post-partum in mice) (White et al. 2010). The cell fusion process underlying this nuclear addition requires regulation of cell migration, recognition, and

adhesion, aspects of which have been reviewed in depth elsewhere (Abmayr and Pavlath 2012). After this developmental timepoint, fibre cross-sectional area continues to increase without the addition of nuclei (White et al. 2010), though hypertrophy via nuclear addition does occur at these later developmental stages when muscle is stimulated via exercise (Bruusgaard et al. 2010). Importantly, regardless of the process used to expand muscle tissue, the mixed nature of myofibre types contributing to the same muscle that is established during embryonic development persists into postnatal and adult muscle growth (Schiaffino and Reggiani 2011). Recent cell lineage ablation studies demonstrating that the main source for nuclei required during growth of skeletal muscle in postnatal life is the Pax7-expressing satellite cell population generated at the embryonic and fetal stages (Gros et al. 2005; Lepper et al. 2009). While many aspects of this process have been elucidated from experiments performed on muscle explants, visualization of these events and the molecular interactions regulating them in an in vivo vertebrate environment can only occur in an organism such as the zebrafish, which remains optically clear throughout its larval development.

Classically, zebrafish are considered to be embryos until they hatch, usually at approximately 72 h post-fertilization (Parichy et al. 2009). Hatchlings are henceforth considered larvae, characterized by swimming and feeding behaviour that becomes readily apparent when these fish inflate their swim bladder and consume their remaining yolk. Adult fish are larvae that have undergone metamorphosis and have acquired mature gametes (Parichy et al. 2009). Using both visual colour and MyHC antibodies as a marker, it becomes readily apparent that slow and fast muscle fibre populations remain segregated throughout larval development and into adulthood. Slow-myosin-expressing oxidative red muscle fibres maintain a superficial layer over the bulk of the fish myotome, which is fast-myosin-expressing glycolytic white muscle (van Raamsdonk et al. 1980; Bone 1975). A small wedge of slow muscle also exists just beneath the lateral line. Finally, most fish species also possess an intermediate, pink-coloured population of fibres possessing intermediate levels of glycolytic and oxidative enzymes and located between the slow and fast layers (van Raamsdonk et al. 1978; Mascarello et al. 1986). Electromyography studies have shown that slow muscle becomes recruited at slow swimming speeds associated with basic locomotion, intermediate muscle becomes recruited at faster cruising speeds associated with feeding and foraging, and fast muscle becomes recruited during burst swimming speeds, associated with predation evasion (Rome et al. 1988; Johnston et al. 1997). Fibre composition depends on the lifestyle of the fish, whereby active foraging species have much higher concentrations of slow-red muscle than sit-and-wait predators (Luther et al. 1995). Therefore, despite the segregation of muscle fibres being a fish-specific condition, these muscle fibres appear to function in a very similar manner to their amniote counterparts.

By contrast to many amniotes, which rely almost exclusively on hypertrophy for muscle growth and expansion, zebrafish and many other fish utilize both hyperplasia and hypertrophy from the moment they hatch and engage swimming and foraging behaviour, the functional equivalent of the postnatal stage in amniotes.

This utilization of hyperplastic growth mechanisms has been demonstrated by simply counting numbers of fibres at various stages of fish growth, which increase as the fish develops (Greer-Walker et al. 1972). Therefore, new skeletal muscle fibres are derived from and produced in different areas of the myotome throughout the life of the fish. This ability to continue producing new fibres results in indeterminate growth and final size of the fish (Bryson-Richardson and Currie, 2008, Rowlerson and Veggetti 2001). Two forms of hyperplasia are generally thought to occur during fish myotome growth—stratified hyperplasia and mosaic hyperplasia. Stratified hyperplasia is defined as the addition of new fibres to a discrete zone of the myotome, along the lateral surface. Mosaic hyperplasia is the expansion of the myotome by addition of muscle fibres throughout the musculature. These two processes were thought to act in a sequential manner, with hypertrophy also playing a part in later fish development (Johnston et al. 2009; Johnston et al. 2011). However, recent work performed on trout shows that fish utilize all three growth processes throughout their development, and what actually varies is the relative input of each process at different developmental stage (Steinbacher et al. 2007) (Fig. 1).

With regards to the cellular population driving the enormous scale of muscle expansion that occurs from early larvae to adult fish, as well as covering the requirement for muscle injury repair, the most likely candidate is the

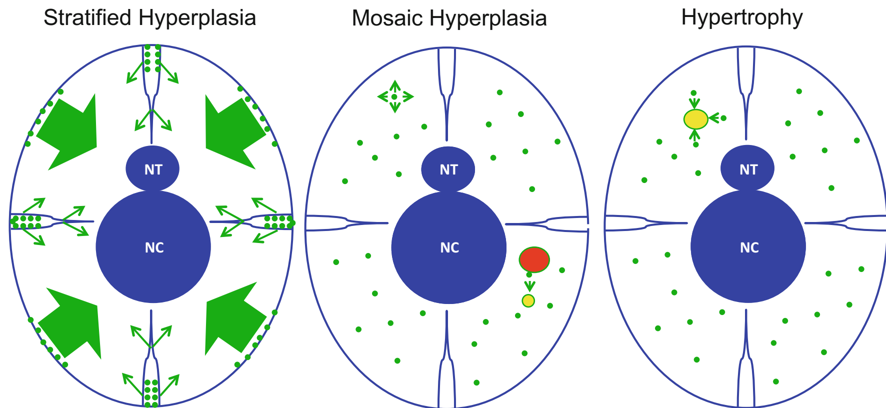


Fig. 1 Cellular mechanisms of myogenesis utilised in post-embryonic zebrafish. Schematic of a transverse section taken through the trunk region of a zebrafish, showing neural tube (NT), notochord (NC), as well as vertical and horizontal myosepta that divide the myotome into quadrants. On the left, the stratified hyperplasia diagram shows addition of new fibres to the myotome from the apical surface and the myosepta (*green circles*). In the centre, the mosaic hyperplasia diagram shows addition of new fibres throughout the myotome, generated by myogenic progenitor cells within the myotome (*green circles*). Some of these progenitor cells are assumed to be associated with mature fibres (*red circle*), as in the mammalian context. On the right, the hypertrophy diagram shows the growth and of immature muscle fibres (*yellow circle*) via fusion of myogenic progenitor cells (*green circles*). While previously it was believed that these mechanisms had discrete timeframes in terms of their contribution to skeletal muscle growth in the fish, it now appears that they all play a continuous, overlapping role throughout development

pax7-expressing lineage (Devoto et al. 2006). Similar to the amniote system, *pax7*-expressing cells have been shown to form muscle fibres in the embryonic and early larval stages (Stellabotte et al. 2007; Seger et al. 2011). Laser capture and micro-array analysis of the active hyperplastic region at embryonic stages in the trout has shown a marked upregulation of *pax7* expression compared to the less hyperplastic adult musculature (Recan et al. 2013). Remnants of the *pax7*-expressing ECL persist well into larval development of zebrafish trunk musculature (Patterson et al. 2008; Devoto et al. 2006; Steinbacher et al. 2011; Hollway et al. 2007), and indeed into adult stages of development (Gurevich et al. in preparation). Isolation of these adult zebrafish skeletal myoblasts reveals a population of cells that express low levels of *pax7* (Alexander et al. 2011; Anderson et al. 2012). During myogenic differentiation in vitro, these cells downregulate early myoblast markers such as *pax7a* and *myf5*, while subsequently upregulating differentiation markers such as *myogenin* and form myotubes in culture (Alexander et al. 2011). These findings support the transferability of zebrafish studies in modelling the role of muscle progenitor cells in juvenile and adult muscle growth.

4 Regeneration and Disease

4.1 Injury Repair

Skeletal muscle is capable of a robust regeneration response when injured. Numerous injury models have been used to investigate this response in amniotes, including physical manipulations such as crush and stab injuries, temperature-based injuries such as heat and freeze injuries, and chemical-based injuries such as those induced by cardiotoxin and barium chloride (Cornelison et al. 2004; d'Albis et al. 1988; Garry et al. 1997). Regardless of the cause of injury, the subsequent process of muscle regeneration progresses in a very similar manner. Once the inflammatory response has been engaged, myogenic cells are activated and migrate into the damaged area (Hawke and Garry 2001), beginning a proliferation response from 1 to 2 days post-injury (McGeachie and Grounds, 1987; Morlet et al., 1989). As in amniote postnatal muscle growth, satellite cells are the main drivers behind the ability of skeletal muscle tissue to completely and repeatedly regenerate, forming both fusion competent myoblasts as well as replenishing their own numbers (Moss and Leblond 1971; Schultz and Jaryszak 1985; Schultz 1996). Satellite cells undergo extensive proliferation within the first 2 to 3 days post-injury, with new centrally nucleated fibres being visible 3–5 days post-injury (Carlson 1973; Garry et al. 1997; Snow 1977). After 5 days post-injury, the satellite cells slow their proliferation and begin to withdraw from the cell cycle (Garry et al. 1997). In the instance of cardiotoxin injuries, the overall architecture is restored within 10–14 days, with nuclei located in a peripheral position as a sign of fibre maturity

(Shi and Garry 2006). In zebrafish, three types of muscle injury have been examined—needle stab, cardiotoxin injection, and laser-induced micro injury (Rowlerson et al. 1997; Otten and Abdelilah-Seyfried 2013; Seger et al. 2011). From these investigations, the pattern of myogenic cell migration, proliferation, fibre formation, and injury resolution described above for amniotes is very similar in the larval zebrafish post needle stab or cardiotoxin injury. Extensive proliferation of myogenic cells is seen in the first 2 days post-injury, and new fibres are seen from 3 days post-injury onwards, with the injury resolving within a little over a week post-injury.

Numerous avenues of investigation have lead to the conclusion that the genetic program of regeneration recapitulates many aspects of earlier muscle development. In the amniote, genetic ablation of satellite cell populations expressing Pax or MRF genes results in interruption or complete abrogation of normal regeneration processes (Lepper et al. 2011; Murphy et al. 2011; Gayraud-Morel et al. 2007). Studies have also shown that all four MRFs are induced in injured muscle tissue within hours post-injury, with *MyoD* and *Mrf4* expression peaking soon after injury and *Myogenin* expression peaking at 3–5 days post-injury, corresponding to the activation and subsequent differentiation of the satellite cell population (Goetsch et al. 2003; Turk et al. 2005; Yan et al. 2003; Launay et al. 2001). In the adult context, loss-of-function *MyoD* delayed but did not prevent adult murine skeletal muscle regeneration (White et al. 2000), while loss-of-function *Myf5* expression results in both delayed and impaired regeneration (Gayraud-Morel et al. 2007). Once again, numerous parallels exist with the zebrafish muscle repair. Previous work has shown that *pax7*-expressing cells are seen in the injury site within 2 days post-needle stab injury, and that *myf5* and *myod* expression has also been detected in newly generated fibres post-injury (Seger et al. 2011).

One important consideration to arise from the muscle damage experiments performed on zebrafish is that muscle repair in adult zebrafish takes up to 42 days (Rowlerson et al. 1997), three to four times longer than in the larvae (Gurevich et al. in preparation) (Seger et al. 2011) or in amniotes (Hawke and Garry 2001). Given that adult muscle injury repair in zebrafish was examined prior to larval muscle injury repair, it was suggested that this extended repair timeframe observed in adult fish compared to adult amniotes is due to the decrease in temperature at which this process occurs—approximately 25 °C in fish compared to 37 °C in amniotes. Clearly, however, this does not hold true for injury repair performed by larval fish. One explanation could be that the myotomal growth and expansion occurring in the larval fish is playing a large role in augmenting the repair process. However, a recent investigation showing that neonatal mice that are actively growing repair their muscle within the same timeframe and using the same cellular response as adult mice (Lepper et al, 2009), suggesting that growth may not be a serious confounding variable at all. Therefore, larval zebrafish may in fact be a more representative model of muscle repair in amniotes, with adult zebrafish incurring an as yet unidentified hindrance in skeletal muscle regenerative mechanics.

4.2 *Myopathies and Muscular Dystrophies*

Numerous muscle disorders exist that affect human health and function, and researchers have focused on establishing models of these to better understand the pathogenesis involved and to potentially identify targets for treatment and amelioration. These disorders are characterized by progressive degeneration of muscle tissue, the mechanisms of which include defects in fibre cytoskeletal structure, the cytoskeletal connection to the extracellular matrix (ECM), the ECM structure itself, or any number of signalling and regulatory events, reviewed extensively in (Rahimov and Kunkel 2013). See Fig. 2 for a diagrammatic representation of some of these important structural components. The drive to use zebrafish as a complement to mammalian models for many of these muscle disorders, and in particular congenital myopathies and muscular dystrophies, stems from the associated secondary defects to the cardiovascular system that lead to embryonic or early postnatal lethality in amniotes. Embryonic and early larval zebrafish can absorb sufficient oxygen through the skin, therefore allowing them to largely bypass the necessity for a functioning cardiovascular system at this developmental timepoint and thus permitting the focused examination of disease pathology on skeletal muscle tissue. Furthermore, the small size, rapid development and high fecundity of zebrafish permits their effective use in forward genetic screening approaches such as large-scale ethylnitrosourea (ENU) and insertional mutagenesis screens, resulting in thousands of mutant alleles for genes involved in motility and muscle

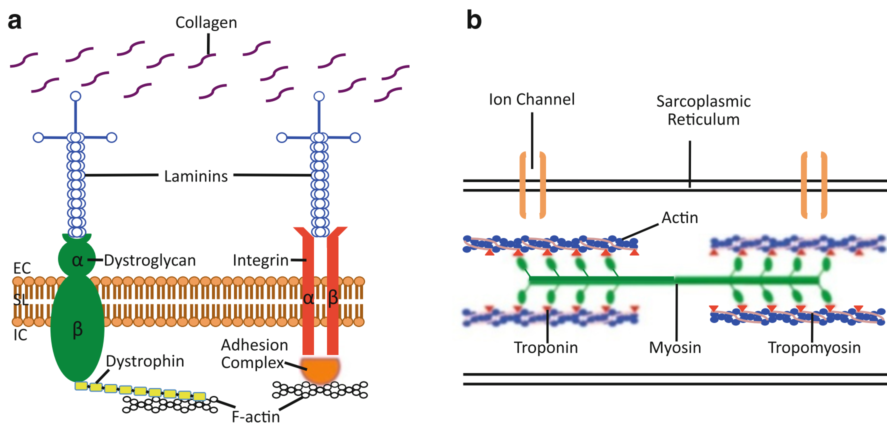


Fig. 2 Simplified schematics of sarcolemmal proteins and sarcomere structure. (**a**) The multimeric Dystrophin-associated glycoprotein complex (DAPC) and integrin dimers are both located on the sarcolemma membrane (SL), and both are involved in linkages between elements of the extracellular matrix (EC), such as laminin and collagen, with the intracellular (IC) located F-actin cytoskeleton. In the case of the DAPC, this linkage is mediated through dystrophin. (**b**) The sarcomere is comprised of thin actin filaments and thick myosin filaments, which can only interact to cause contraction once the troponin/tropomyosin complex has been removed via ion channel-mediated calcium ion influx

structure (Granato and Nüsslein-Volhard 1996; Haffter et al. 1996). Coupled with their previously stated advantages of optical clarity and genetic tractability, these traits allow the use of zebrafish in investigating the cellular and molecular mechanisms underlying these muscle diseases.

A major focus has been the modelling of dystrophies of defects to either the integrin-associated complex or the dystrophin-associated glycoprotein complex. These two complexes are comprised of distinct groups of proteins that provide a structural and mechanical link between the muscle fibre cytoskeleton and the extracellular matrix, responsible for transferring the generated contractile force as well as membrane integrity and cell signalling. Comprehensive reviews for the various molecular players that comprise these complexes have been presented elsewhere (Mercuri and Muntoni 2013). In brief, genetic deficits for various components of these complexes, such as the dystrophin gene, result in the most common human myopathies—a complete loss of dystrophin results in the severe Duchenne Muscular Dystrophy (DMD), while a partial loss of dystrophin results in the milder Becker Muscular Dystrophy (BMD). A dystrophin-deficient mdx mouse has been identified, however this model has been shown to possess a very mild phenotype, lacking the severity and common symptoms involved in the human progression of the disease such as repeated rounds of inflammation and fibrosis (Sicinski et al. 1989).

Initial investigation of the zebrafish *dystrophin* mutant allele *sapje* (sap^{ta222a}) revealed a complete lack of Dystrophin immunoreactivity isolated to muscle tissue only, indicating that only muscle-specific isoforms of Dystrophin are affected (Bassett et al. 2003). These fish were identified by their limited escape response, a typical reaction consisting of spontaneous muscle contraction upon mechanical stimulation that is seen at day three post-fertilization in wild-type fish (Granato et al. 1996). Further study of the *sapje* allele indicated a consistency with human DMD disease pathology, such as continuous and increasing muscle fibre detachment coupled with necrosis, inflammation, fibrosis, and mortality at relatively early developmental stages (Berger et al. 2010). The progression of these symptoms and the resultant myofibre integrity could be directly visualized and quantified in the embryonic and larval fish due to the birefringence phenomenon, the property of intact muscle tissue to diffract polarized light, as opposed to damaged muscle tissue that lacks this quality (Berger et al. 2012). This optical clarity also allowed for an identification of sarcolemmal rupture as the mechanism underlying the fibre detachment in this disease model (Bassett et al. 2003). Furthermore, the ease of administering and removing anaesthetic to immobilized fish demonstrated that these detachments occur as a result of muscle fibre contraction (Berger et al. 2010). Other transmembrane proteins have also attracted attention due to being implicated in human dystrophies. As an example, the protein Dysferlin is involved in repair of sarcolemmal damage that occurs due to mechanical stress, and deficits in its expression have been specifically linked to Limb-Girdle Muscular Dystrophy and Myoshi Myopathy (Bansal et al. 2003; Bashir et al. 1998; Liu et al. 1998). Modelling this disease in zebrafish resulted in muscle abnormalities similar to those seen in humans, and examination of the specific mechanism involved in this type of

sarcolemmal repair revealed novel insights into this process, implicating cytoplasimic annexin molecules to the injury site (Roostalu and Strähle 2012). These investigations show the utility of the zebrafish in studying molecular examinations in real time and in vivo, as well as demonstrating superiority in modelling many aspects of disease progression compared with mouse.

By contrast, mutations in components of the basement membrane that interact with these structural complexes from the extracellular environment—such as the interaction with laminins—have been detected in a different type of congenital muscular dystrophy (Helbling-Leclerc et al. 1995; Allamand et al. 1997). Complete or partial loss of laminin a2 (LAMA2) again leads to a severe (MDC1A) or milder (LGMD) form of muscular dystrophy, respectively, and this has also been modelled in mouse (Guo et al. 2003). Identification of mutated zebrafish *lama2 candyfloss* allele was performed via the escape response motility assay described previously, as well as reduced skeletal muscle expression of *lama2*. In this model, however, detached muscle fibres appeared to maintain sarcolemmal integrity, with the structural deficit occurring within the basement membrane (Hall et al. 2007). Finally, muscle fibre death was delayed in the *candyfloss* muscle dystrophy model compared to *sapje*, suggesting that the differing structural deficits were resulting in two separate mechanisms of cell death (Hall et al. 2007). A further mutated laminin, the lamininb2 *softy* zebrafish mutant, presents with a basement membrane deficit that is non-lethal, allowing the detached muscle fibres to reattach to ectopic basement membrane and thus remain viable (Jacoby et al. 2009). Mutations in genes responsible for other ECM components, such as collagen, can also result in muscle disease, specifically Ullrich congenital Muscular Dystrophy or Bethlem Myopathy (Camacho Vanegas et al. 2001; Jöbsis et al. 1996). In humans, these diseases result in a spectrum of symptoms ranging from severe muscle weakness in the former disease to more mild and progressive muscle weakness in the latter. While a mutant *Col6a1* collagen mouse does exist, it presents with only a mild phenotype (Bonaldo et al. 1998), whereas both mild and severe phenotypes have been modelled in the fish (Telfer et al. 2010). Together, these investigations into fish deficient in structural transmembrane or ECM components have yielded an understanding of numerous mechanisms underlying these congenital myopathies and suggest various targets for therapeutic strategies.

The sarcomere is another structure that appears to be often affected in genetic disorders of skeletal muscle. This apparatus is the basic unit underlying muscle contraction and is composed of a highly ordered arrangement of thick and thin cables of myosin or actin, troponin, and tropomyosin, respectively. In response to depolarization of the adjacent membrane structures at the moment of activation, specialized Ca^{2+} ion channels and receptors—such as the ryanodine receptor 1 (RyR1)—are utilized, resulting in an influx of cytoplasmic Ca^{2+} that in turn temporarily removes the troponin/tropomyosin complex and allows the actin–myosin sliding required for muscle contraction. In humans, mutation of the RYR1 gene results in numerous muscular disorders (Kaplan 2011), and zebrafish *ryr*/*ryr1b* mutants displays many similarities in phenotype, including weak muscle contractions, compromised Ca^{2+} handling post-stimulation, and formation of small

amorphous cores throughout the muscle, deficits that were corrected upon restoration of normal splicing (Hirata et al. 2007). Defects in other contraction-associated proteins also lead to disease, with mutations in the human fast muscle-specific troponin TNNT3 gene resulting in muscle fibrosis and muscle shortening, while mutations in cardiac muscle-specific TNNC1 and TNNT2 are associated with hypertrophic cardiomyopathy and sudden cardiac death (Kaplan 2011). Here too, malfunctions in analogous zebrafish genes result in similar deficits. Investigations of the zebrafish *tnni2a.4* mutant affecting fast muscle troponin suggested that the observed loss of muscle function is due to decreased myofibril integrity and progressive paralysis via sarcomeric disintegration (Ferrante et al. 2011). Mutations in *tnnt2a* affecting cardiac-specific troponin lead to a non-contractile heart phenotype known as *silent heart*, where cellular excitation in cardiomyocytes functions normally but sarcomeric assembly is defective (Sehnert et al. 2002). Work on zebrafish mutants has shown that even the correct folding of contractile elements such as myosin is important for myofibrillogenesis and subsequent muscle function, with mutations in the molecular chaperones Hsp90 and Unc45b resulting in disorganized sarcomeres lacking in thick filaments and severely compromised muscle function dependent on the specific expression of particular alleles (Etard et al. 2007; Hawkins et al. 2008).

Numerous other zebrafish models of human myopathies exist, and have been reviewed elsewhere (Gibbs et al. 2013). To date, most of these have been generated via the previously mentioned mutagenesis-based forward screens, or alternatively via morpholino-based knockdown using synthetic oligonucleotides that inhibit translation or pre-processing of mRNA, resulting in a transient “morphant” fish lacking protein expression of the gene of interest. These approaches can be highly time consuming in the case of mutagenesis screens or have numerous off-target effects and only affect the first few days of development in the case of morpholino injections. Recently, more sophisticated genome editing technology has emerged that allows the targeted and permanent inactivation of a gene to investigate the resultant phenotype, known as reverse genetics. Zinc finger nucleases (ZFN) and transcription activator-like effector nucleases (TALENs) have been shown to introduce double-strand breaks in DNA targeted to precise genomic locations, thereby generating somatic and germline mutations (Ekker 2008; Sander et al. 2011). Most recently, a third gene-editing technology known as Clustered Regularly Interspaced Short Palindromic Repeats (CRISPRs) has become available as a more specific and higher efficiency alternative to ZFNs and TALENs, resulting in controlled insertions and deletions that are heritable and rarely affect off-target sites (Hruscha et al. 2013; Chang et al. 2013). High-throughput sequencing and screening has also allowed for an improvement in large-scale chemical mutagenesis to produce the methodology of Targeting Induced Local Lesions IN Genomes (TILLING), in order to identify mutations in specific genes of interest (Kettleborough et al. 2011). Finally, the ability of zebrafish embryos to survive in small volumes of liquid and absorb various chemical compounds directly through their skin allows their use in drug efficacy screening. Using a high-throughput, automated visual screening platform such as the Viewpoint Zebrabox, the

swimming behaviours of wild-type and mutant fish can provide a simple but robust measure of muscle function, permitting the testing of many new therapeutic interventions (Gibbs et al 2013). Indeed, there is already an example of large chemical library screening yielding a new candidate therapeutic drug for the restoration of muscle tissue in *sapje* mutant zebrafish by utilizing birefringence as a readout of muscle tissue integrity (Kawahara et al. 2011). Importantly, this candidate drug has already been shown to ameliorate heart defects associated with mouse DMD models, suggesting that future drug compounds identified in the zebrafish are likely to be relevant in a mammalian context (Adamo et al. 2010).

In conclusion, zebrafish represent a powerful complement to mammalian models of both muscle development and myopathies. Combining the recent advances in our understanding of the muscle stem cell activation and function in vivo during growth and repair with zebrafish models of disease, we have a relevant and transferable paradigm in dissecting mechanisms of many myopathies. Ultimately, by further understanding the behaviour of myogenic cells underlying growth and repair, we hope to be able to elucidate treatment strategies for acute conditions, such as severe traumatic injuries of the muscle, and chronic conditions, such DMD or sarcopenia.

References

Abmayr SM, Pavlath GK (2012) Myoblast fusion: lessons from flies and mice. Development 139:641–656

Adamo CM, Dai DF, Percival JM, Minami E, Willis MS, Patrucco E, Froehner SC, Beavo JA (2010) Sildenafil reverses cardiac dysfunction in the mdx mouse model of Duchenne muscular dystrophy. Proc Natl Acad Sci U S A 107:19079–19083

Alexander MS, Kawahara G, Kho AT, Howell MH, Pusack TJ, Myers JA, Montanaro F, Zon LI, Guyon JR, Kunkel LM (2011) Isolation and transcriptome analysis of adult zebrafish cells enriched for skeletal muscle progenitors. Muscle Nerve 43(5):741–750

Allamand V, Sunada Y, Salih MA, Straub V, Ozo CO, Al-Turaiki MH, Akbar M, Kolo T, Colognato H, Zhang X, Sorokin LM, Yurchenco PD, Tryggvason K, Campbell KP (1997) Mild congenital muscular dystrophy in two patients with an internally deleted laminin alpha2-chain. Hum Mol Genet 6(5):747–752

Andermarcher E, Surani MA, Gherardi E (1996) Co-expression of the HGF/SF and c-met genes during early mouse embryogenesis precedes reciprocal expression in adjacent tissues during organogenesis. Dev Genet 18:254–266

Anderson JE, Wozniak AC, Mizunoya W (2012) Single muscle-fiber isolation and culture for cellular, molecular, pharmacological, and evolutionary studies. Methods Mol Biol 798:85–102

Arnold HH, Braun T (1996) Targeted inactivation of myogenic factor genes reveals their role during mouse myogenesis: a review. Int J Dev Biol 40:345–353

Bajard L, Relaix F, Lagha M, Rocancourt D, Daubas P, Buckingham ME (2006) A novel genetic hierarchy functions during hypaxial myogenesis: Pax3 directly activates Myf5 in muscle progenitor cells in the limb. Genes Dev 20:2450–2464

Bansal D, Miyake K, Vogel SS, Groh S, Chen CC, Williamson R, McNeil PL, Campbell KP (2003) Defective membrane repair in dysferlindeficient muscular dystrophy. Nature 423:168–172

Bashir R, Britton S, Strachan T, Keers S, Vafiadaki E, Lako M, Richard I, Marchand S, Bourg N, Argov Z, Sadeh M, Mahjneh I, Marconi G, Passos-Bueno MR, Moreira ES, Zatz M, Beckmann

JS, Bushby K (1998) A gene related to Caenorhabditis elegans spermatogenesis factor fer-1is mutated in limb-girdle muscular dystrophy type 2B. Nat Genet 20:37–42
Bassett DI, Bryson-Richardson RJ, Daggett DF, Gautier P, Keenan DG, Currie PD (2003) Dystrophin is required for the formation of stable muscle attachments in the zebrafish embryo. Development 130(25):5851–5860
Ben-Yair R, Kalcheim C (2005) Lineage analysis of the avion dermomyotome sheet reveals the existence of single cells with both dermal and muscle progenitor fates. Development 132:689–701
Berger J, Berger S, Hall TE, Lieschke GJ, Currie PD (2010) Dystrophin-deficient zebrafish feature aspects of the Duchenne muscular dystrophy pathology. Neuromuscul Disord 20:826–32
Berger J, Sztal T, Currie PD (2012) Quantification of birefringence readily measures the level of muscle damage in zebrafish. Biochem Biophys Res Commun 423:785–788
Blagden CS, Currie PD, Ingham PW, Hughes SM (1997) Notochord induction of zebrafish slow muscle mediated by Sonic hedgehog. Genes Dev 11:2163–2175
Bober E, Lyons GE, Braun T, Cossu G, Buckingham M, Arnold HH (1991) The muscle regulatory gene, Myf-6, has a biphasic pattern of expression during early mouse development. J Cell Biol 113:1255–1265
Bonaldo P, Braghetta P, Zanetti M, Piccolo S, Volpin D, Bressan GM (1998) Collagen VI deficiency induces early onset myopathy in the mouse: an animal model for Bethlem myopathy. Mol Genet 7:2135–2140
Bone Q (1975) Muscular and energetic aspects of fish swimming. Swimming Flying Nature 2:493–528
Bone Q (1989) Evolutionary patterns of axial muscle systems in some invertebrates and fish. Am Zool 29:5–18
Brand-Saberi B, Christ B (2000) Evolution and development of distinct cell lineages derived from somites. Curr Top Dev Biol 48:1–42
Braun T, Gautel M (2011) Transcriptional mechanisms regulating skeletal muscle differentiation, growth and homeostasis. Nat Rev 12:349–361
Braun T, Bober E, Winter B, Rosenthal N, Arnold HH (1990) Myf-6, a new member of the human gene family of myogenic determination factors: evidence for a gene cluster on chromosome 12. EMBO J 9:821–831
Bruusgaard JC, Johansen IB, Egner IM, Rana ZA, Gundersen K (2010) Myonuclei acquired by overload exercise precede hypertrophy and are not lost on detraining. Proc Natl Acad Sci U S A 107(34):15111–15116
Bryson-Richardson RJ, Currie PD (2008) The genetics of vertebrate myogenesis. Nat Rev Genet 9 (8):632–646. doi:10.1038/nrg2369
Bryson-Richardson RJ, Daggett DF, Cortes F, Neyt C, Keenan DG, Currie PD (2005) Myosin heavy chain expression in zebrafish and slow muscle composition. Dev Dyn 233:1018–1022
Camacho Vanegas O, Bertini E, Zhang RZ, Petrini S, Minosse C, Sabatelli P, Giusti B, Chu ML, Pepe G (2001) Ullrich scleroatonic muscular dystrophy is caused by recessive mutations in collagen type VI. Proc Natl Acad Sci U S A 98(7516–7521)
Carlson BM (1973) The regeneration of skeletal muscle. A review. Am J Anat 137(2):119–149
Chang N, Sun C, Gao L, Zhu D, Xu X, Zhu X, Xiong JW, Xi JJ (2013) Genome editing with RNA-guided Cas9 nuclease in zebrafish embryos. Cell Res 23:465–472
Cinnamon Y, Kahane N, Kalcheim C (1999) Characterization of the early development of specific hypaxial muscles from the ventrolateral myotome. Development 126:4305–4315
Cornelison DD (2008) Context matters: in vivo and in vitro influences on muscle satellite cell activity. J Cell Biochem 105:663–669
Cornelison DD, Wold BJ (1997) Single-cell analysis of regulatory gene expression in quiescent and activated mouse skeletal muscle satellite cells. Dev Biol 191:270–283
Cornelison DD, Wilcox-Adelman SA, Goetinck PF, Rauvala H, Rapraeger AC, Olwin BB (2004) Essential and separable roles for Syndecan-3 and Syndecan-4 in skeletal muscle development and regeneration. Genes Dev 18:2231–2236

Cossu G, De Angelis L, Borello U, Beraducci B, Buffa V, Sonnino C, Coletta M, Vivarelli E, Bouche M, Lattanzi L, Tosoni D, Di Donna S, Berghella L, Salvatori G, Murphy P, Cusella-de Angelis MG, Molinaro M (2000) Determination, diversification and multipotency of mammalian myogenic cells. Int J Dev Biol 44:699–706

Coutelle O, Blagden CS, Hampson R, Halai C, Rigby PW, Hughes SM (2001) Hedgehog signalling is required for maintenance of myf5 and myoD expression and timely terminal differentiation in zebrafish adaxial myogenesis. Dev Biol 236:136–150

Currie PD, Ingham PW (1996) Induction of a specific muscle cell type by a hedgehoglike protein in zebrafish. Nature 382:452–455

Currie PD, Ingham PW (2001) Embryonic skeletal muscle in the zebrafish. Muscle growth and development. Academic, NY

d'Albis A, Couteaux R, Janmot C, Roulet A, Mira JC (1988) Regeneration after cardiotoxin injury of innervated and denervated slow and fast muscles of mammals. Myosin isoform analysis. Eur J Biochem 174:103–110

Davis TA, Fiorotto ML (2009) Regulation of muscle growth in neonates. Curr Opin Clin Nutr Metab Care 12(1):78–85

Davis RL, Weintraub H, Lassar AB (1987) Expression of a single transfected cDNA converts fibroblasts to myoblasts. Cell 51:987–1000

Denetclaw WF, Ordahl CP (2000) The growth of the dermomyotome and formation of early myotome lineages in thoracolumbar somites of chicken embryos. Development 127:893–905

Deries M, Collins JJ, Duxson MJ (2008) The mammalian myotome: a muscle with no innervation. Evol Dev 10(6):746–755

Deries M, Schweitzer R, Duxson M (2010) Developmental fate of the mammalian myotome. Dev Dyn 239:2898–2910

Devoto SH, Melancon E, Eisen JS, Westerfield M (1996) Identification of separate slow and fast muscle precursor cells in vivo, prior to somite formation. Development 122:3371–3380

Devoto SH, Stoiber W, Hammond CL, Steinbacher P, Haslett JR, Barresi MJ, Patterson SE, Adiarte EG, Hughes SM (2006) Generality of vertebrate developmental patterns: evidence for a dermomyotome in fish. Evol Dev 8(1):101–110. doi:10.1111/j.1525-142X.2006.05079.x

Dolez M, Nicolas JF, Hirsinger E (2011) Laminins, via heparan sulfate proteoglycans, participate in zebrafish myotome morphogenesis by modulating the pattern of BMP responsiveness. Development 138(1)

Downs KM, Davies T (1993) Staging of gastrulating mouse embryos by morphological landmarks in the dissecting microscope. Development 118:1255–1266

Du M, Tong J, Zhao J, Underwood KR, Zhu M, Ford SP, Nathanielsz PW (2010) Fetal programming of skeletal muscle development in ruminant animals. J Anim Sci 88:E51–60

Edmondson DG, Olson EN (1989) A gene with homology to the myc similarity region of MyoD1 is expressed during myogenesis and is sufficient to activate the muscle differentiation program. Genes Dev 3:628–640

Ekker SC (2008) Zinc finger-based knockout punches for zebrafish genes. Zebrafish 5:121–123

Enesco M, Puddy D (1964) Increase in the number of nuclei and weight in skeletal muscle of rats of various ages. Am J Anat 114:235–244

Etard C, Behra M, Fischer N, Hutcheson D, Geisler R, Strahle U (2007) The UCS factor Steif/Unc-45b interacts with the heat shock protein Hsp90a during myofibrillogenesis. Dev Biol 308:133–143

Fairclough RJ, Bareja A, Davies KE (2011) Progress in therapy for Duchenne muscular dystrophy. Exp Physiol 96:1101–1113

Felsenfeld AL, Curry M, Kimmel CB (1991) The fub-1 mutation blocks initial myofibril formation in the zebrafish muscle pioneer cells. Dev Biol 148:23–30

Ferrante MI, Kiff RM, Goulding DA, Stemple DL (2011) Troponin T is essential for sarcomere assembly in zebrafish skeletal muscle. J Cell Sci 124:565–577

Figeac N, Daczewska M, Marcelle C, Jagla K (2007) Muscle stem cells and model systems for their investigation. Dev Dyn 236:3332–3342

Frontera WR, Zayas AR, Rodriguez N (2012) Aging of human muscle: understanding sarcopenia at the single muscle cell level. Phys Med Rehabil Clin N Am 23(1):201–207

Garry DJ, Yang Q, Bassel-Duby R, Williams RS (1997) Persistent expression of MNF identifies myogenic stem cells in postnatal muscles. Dev Biol 188:280–294

Gayraud-Morel B, Chrétien F, Flamant P, Gomès D, Zammit PS, Tajbakhsh S (2007) A role for the myogenic determination gene Myf5 in adult regenerative myogenesis. Dev Cell 312(1):13–28

Gibbs EM, Horstick EJ, Dowling JJ (2013) Swimming into prominence: the zebrafish as a valuable tool for studying human myopathies and muscular dystrophies. FEBS J 280(17):4187–4197

Goetsch SC, Hawke TJ, Gallardo TD, Richardson JA, Garry DJ (2003) Transcriptional profiling and regulation of the extracellular matrix during muscle regeneration. Physiol Genomics 14:261–271

Goldsmith JR, Jobin C (2012) Think small: Zebrafish as a model systemof human pathology. J Biomed Biotechnol 2012, 817341

Goulding MD, Chalepakis G, Deutsch U, Erselius JR, Gruss P (1991) Pax-3, a novel murine DNA binding protein expressed during early neurogenesis. EMBO J 10:1135–1147

Granato M, Nüsslein-Volhard C (1996) Fishing for genes controlling development. Curr Opin Genet Dev 6(4):461–468

Granato M, van Eeden FJ, Schach U, Trowe T, Brand M, Furutani-Seiki M, Haffter P, Hammerschmidt M, Heisenberg CP, Jiang YJ, Kane DA, Kelsh RN, Mullins MC, Odenthal J, Nüsslein-Volhard C (1996) Genes controlling and mediating locomotion behavior of the zebrafish embryo and larva. Development 123:399–413

Greer-Walker M, Burd AC, Pull GA (1972) The total number of white skeletal muscl fibres in cross section as a character for stock separation in North sea herring (*Clupea harengus*). J Cons Int Explor Mer 34:238–243

Gros J, Scaal M, Marcelle C (2004) A two-step mechanism for myotome formation in chick. Dev Cell 6:875–882

Gros J, Manceau M, Thome V, Marcelle C (2005) A common somitic origin for embryonic muscle progenitors and satellite cells. Nature 435:954–958

Groves J, Hammond C, Hughes SM (2005) Fgf8 drives myogenic progression of a novel lateral fast muscle fibre population in zebrafish. Development 132(19):4211–4222

Guo LT, Zhang XU, Kuang W, Xu H, Liu LA, Vilquin JT, Miyagoe-Suzuki Y, Takeda S, Ruegg MA, Wewer UM, Engvall E (2003) Laminin alpha2 deficiency and muscular dystrophy; genotype-phenotype correlation in mutant mice. Neuromuscul Disord 13(3):207–215

Guyon JR, Steffen LS, Howell MH, Pusack TJ, Lawrence C, Kunkel LM (2007) Modeling human muscle disease in zebrafish. Biochim Biophys Acta 1772(2):205–215

Haffter P, Granato M, Brand M, Mullins MC, Hammerschmidt M, Kane DA, Odenthal J, van Eeden FJ, Jiang YJ, Heisenberg CP, Kelsh RN, Furutani-Seiki M, Vogelsang E, Beuchle D, Schach U, Fabian C, Nüsslein-Volhard C (1996) The identification of genes with unique and essential functions in the development of the zebrafish, Danio rerio. Development 123:1–36

Hall TE, Bryson-Richardson RJ, Berger S, Jacoby AS, Cole NJ, Hollway GE, Berger J, Currie PD (2007) The zebrafish candyfloss mutant implicates extracellular matrix adhesion failure in laminin alpha2-deficient congenital muscular dystrophy. Proc Natl Acad Sci USA 104 (17):7092–7097. doi:10.1073/pnas.0700942104

Hamade A, Deries M, Begemann G, Bally-Cuif L, Genet C, Sabatier F, Bonnieu A, Cousin X (2006) Retinoic acid activates myogenesis in vivo through Fgf8 signalling. Dev Biol 289:127–140

Hamburger V, Hamilton HL (1951) A series of normal stages in the development of the chick embryo. Dev Dyn 195:231–272

Hammond CL, Hinits Y, Osborn DP, Minchin JE, Tettamanti G, Hughes SM (2007) Signals and myogenic regulatory factors restrict pax3 and pax7 expression to dermomyotome-like tissue in zebrafish. Dev Biol 302(2):504–521. doi:10.1016/j.ydbio.2006.10.009

Hasty P, Bradley A, Morris JH, Edmondson DG, Venuti JM, Olson EN, Klein WH (1993) Muscle deficiency and neonatal death in mice with a targeted mutation in the myogenin gene. Nature 364(6437):501–506. doi:10.1038/364501a0

Hawke TJ, Garry DJ (2001) Myogenic satellite cells: physiology to molecular biology. J Appl Physiol 91:534–551

Hawkins TA, Haramis AP, Etard C, Prodromou C, Vaughan CK, Ashworth R, Ray S, Behra M, Holder N, Talbot WS, Pearl LH, Strähle U, Wilson SW (2008) The ATPase-dependent chaperoning activity of Hsp90a regulates thick filament formation and integration during skeletal muscle myofibrillogenesis. Development 135:1147–1156

Helbling-Leclerc A, Zhang X, Topaloglu H, Cruaud C, Tesson F, Weissenbach J, Tomé FM, Schwartz K, Fardeau M, Tryggvason K (1995) Mutations in the laminin alpha 2-chain gene (LAMA2) cause merosin-deficient congenital muscular dystrophy. Nat Genet 11(2):216–218

Helenius IT, Yeh JR (2012) Small zebrafish in a big chemical pond. J Cell Biochem 113 (7):2208–2216

Henry CA, Amacher SL (2004) Zebrafish slow muscle cell migration induces a wave of fast muscle morphogenesis. Dev Cell 7:917–923

Hinits Y, Osborn DPS, Carvajal JJ, Rigby PWJ, Hughes SM (2007) Mrf4 (myf6) is dynamically expressed in differentiated zebrafish skeletal muscle. Gene Expr Patterns 7:738–745

Hinits Y, Osborn DPS, Hughes SM (2009) Differential requirements for myogenic regulatory factors distinguish medial and lateral somitic, cranial and fin muscle fibre populations. Development 136:403–414

Hinits Y, Williams VC, Sweetman D, Donn TM, Ma TP, Moens CB, Hughes SM (2011) Defective cranial development, larval lethality and haploinsufficiency in Myod mutant zebrafish. Dev Biol 358(1):102–112

Hinterberger TJ, Sassoon DA, Rhodes SJ, Konieczny SF (1991) Expression of the muscle regulatory factor MRF4 during somite and skeletal myofiber development. Dev Biol 147:144–156

Hirata H, Watanabe T, Hatakeyama J, Sprague SM, Saint-Amant L, Nagashima A, Cui WW, Zhou W, Kuwada JY (2007) Zebrafish relatively relaxed mutants have a ryanodine receptor defect, show slow swimming and provide a model of multi-minicore disease. Development 134:2771–2781

Holley SA (2007) The Genetics and Embryology of Zebrafish Metamerism. Dev Dyn 236:1422–1449

Hollway GE, Bryson-Richardson RJ, Berger S, Cole NJ, Hall TE, Currie PD (2007) Whole-somite rotation generates muscle progenitor cell compartments in the developing zebrafish embryo. Dev Cell 12(2):207–219. doi:10.1016/j.devcel.2007.01.001

Horst D, Ustanina S, Sergi C, Mikuz G, Juergens H, Braun T, Vorobyov E (2006) Comparative expression analysis of Pax3 and Pax7 during mouse myogenesis. Int J Dev Biol 50:47–54

Hruscha A, Krawitz P, Rechenberg A, Heinrich V, Hecht J, Haass C, Schmid B (2013) Efficient CRISPR/Cas9 genome editing with low off-target effects in zebrafish. Development 140 (24):4982–4987

Hu P, Geles KG, Paik JH, DePinho RA, Tjian R (2008) Codependent activators direct myoblast specific MyoD transcription. Dev Cell 15:534–546

Hutcheson DA, Zhao J, Merrell A, Haldar M, Kardon G (2009) Embryonic and fetal limb myogenic cells are derived from developmentally distinct progenitors and have different requirements for b-catenin. Genes Dev 23:997–1013

Jacoby AS, Busch-Nentwich E, Bryson-Richardson RJ, Hall TE, Berger J, Berger S, Sonntag C, Sachs C, Geisler R, Stemple DL, Currie PD (2009) The zebrafish dystrophic mutant softy maintains muscle fibre viability despite basement membrane rupture and muscle detachment. Development 136(19):3367–3376

Jöbsis GJ, Keizers H, Vreijling JP, de Visser M, Speer MC, Wolterman RA, Baas F, Bolhuis PA (1996) Type VI collagen mutations in Bethlem myopathy, an autosomal dominant myopathy with contractures. Nat Genet 14:113–115

Johnston IA, Cole NJ, Vieira VLA, Davidson I (1997) Temperature and developmental plasticity of muscle phenotype in herring larvae. J Exp Biol 200:849–868

Johnston IA, Lee H-T, Macqueen DJ, Paranthaman K, Kawashima C, Anwar A, Kinghorn JR, Dalmay T (2009) Embryonic temperature affects muscle fibre recruitment in adult zebrafish: genome-wide changes in gene and microRNA expression associated with the transition from hyperplastic to hypertrophic growth phenotypes. J Exp Biol 212:1781–1793

Johnston IA, Bower NI, Macqueen DJ (2011) Growth and the regulation of myotomal muscle mass in teleost fish. J Exp Biol 214:1617–1628

Jostes B, Walther C, Gruss P (1990) The murine paired box gene, Pax7, is expressed specifically during the development of the nervous and muscular system. Mech Dev 33:27–37

Kahane N, Cinnamon Y, Kalcheim C (1998) The origin and fate of pioneer myotomal cells in the avian embryo. Mech Dev 74:59–73

Kahane N, Cinnamon Y, Kalcheim C (2002) The roles of cell migration and myofiber intercalation in patterning formation of the postmitotic myotome. Development 129:2675–2687

Kalcheim C, Ben-Yair R (2005) Cell rearrangements during development of the somite and its derivatives. Curr Opin Genet Dev 15:371–380

Kalcheim C, Cinnamon Y, Kahane N (1999) Myotome formation: a multistage process. Cell Tissue Res 296:161–173

Kaplan JC (2011) The 2012 version of the gene table of monogenic neuromuscular disorders. Neuromuscul Disord 21:833–861

Kassar-Duchossoy L, Gayraud-Morel B, Gomes D, Rocancourt D, Buckingham M, Shinin V, Tajbakhsh S (2004) Mrf4 determines skeletal muscle identity in Myf5:Myod double-mutant mice. Nature 431:466–471

Kawahara G, Karpf JA, Myers JA, Alexander MS, Guyon JR, Kunkel LM (2011) Drug screening in a zebrafish model of Duchenne muscular dystrophy. Proc Natl Acad Sci U S A 108 (13):5331–5336

Kettleborough RN, Bruijn E, Eeden F, Cuppen E, Stemple DL (2011) High-throughput target-selected gene inactivation in zebrafish. Methods Cell Biol 104:121–127

Kiefer JC, Hauschka SD (2001) Myf-5 is transiently expressed in nonmuscle mesoderm and exhibits dynamic regional changes within the presegmented mesoderm and somites I-IV. Dev Biol 232:77–90

Kimmel CB, Ballard WW, Kimmel SR, Ullmann B, Schilling TF (1995) Stages of embryonic development of the Zebrafish. Dev Dyn 203:253–310

Kobiyama A, Nihei Y, Hirayama Y, Kikuchi Y, Suetake H, Johnston IA, Watabe S (1998) Molecular cloning and developmental expression patterns of the MyoD and MEF2 families of muscle transcription factors in the carp. J Exp Biol 201:2801–2813

Launay T, Armand AS, Charbonnier F, Mira JC, Donsez E, Gallien CL, Chanoine C (2001) Expression and neural control of myogenic regulatory factor genes during regeneration of mouse soleus. J Histochem Cytochem 49(7):887–899

Lepper C, Fan CM (2010) Inducible lineage tracing of Pax7-descendant cells reveals embryonic origin of adult satellite cells. Genesis 48:424–436

Lepper C, Conway SJ, Fan CM (2009) Adult satellite cells and embryonic muscle progenitors have distinct genetic requirements. Nature 460:627–631

Lepper C, Partridge TA, Fan CM (2011) An absolute requirement for Pax7-positive satellite cells in acute injury-induced skeletal muscle regeneration. Development 138:3639–3646

Liu J, Aoki M, Illa I, Wu C, Fardeau M, Angelini C, Serrano C, Urtizberea JA, Hentati F, Hamida MB, Bohlega S, Culper EJ, Amato AA, Bossie K, Oeltjen J, Bejaoui K, McKenna-Yasek D, Hosler BA, Schurr E, Arahata K, de Jong PJ, Brown RHJ (1998) Dysferlin, a novel skeletal muscle gene, is mutated in Miyoshi myopathy and limb girdle muscular dystrophy. Nat Genet 20:31–36

Luther PK, Munro LJ, Squire JM (1995) Muscle ultrastructure in the teleost fish. Micron 26:431–459

Marschallinger J, Obermayer A, Sänger AM, Stoiber W, Steinbacher P (2009) Postembryonic fast muscle growth of teleost fish depends upon a nonuniformly distributed population of mitotically active Pax7+ precursor cells. Dev Dyn 238(9):2442–2448

Mascarello F, Romanello MG, Scapolo PA (1986) Histochemical and immunohistochemical profile of pink muscle fibres in some teleosts. Histochemistry 84:251–255

Maurya AK, Tan H, Souren M, Wang X, Wittbrodt J, Ingham PW (2011) Integration of Hedgehog and BMP signalling by the engrailed2A gene in the zebrafish myotome. Development 138 (4):755–765

McGeachie JK, Grounds MD (1987) Initiation and duration of muscle precursor replication after mild and severe injury to skeletal muscle of mice. An autoradiographic study. Cell Tissue Res 248(1):125–130

Mercuri E, Muntoni F (2013) Muscular dystrophies. Lancet 381:845–860

Miner JH, Wold B (1990) Herculin, a fourth member of the MyoD family of myogenic regulatory genes. Proc Natl Acad Sci U S A 87:1089–1093

Morin-Kensicki EM, Eisen JS (1997) Sclerotome development and peripheral nervous system segmentation in embryonic zebrafish. Development 124:159–167

Morlet K, Grounds MD, McGeachie JK (1989) Muscle precursor replication after repeated regeneration of skeletal muscle in mice. Anat Embryol (Berl) 180(5):471–478

Moss FP, Leblond CP (1971) Satellite cells as the source of nuclei in muscles of growing rats. Anat Rec 170:421–435

Murphy M, Kardon G (2011) Origin of vertebrate limb muscle: the role of progenitor and myoblast populations. Curr Top Dev Biol 96:1–32

Murphy MM, Lawson JA, Mathew SJ, Hutcheson DA, Kardon G (2011) Satellite cells, connective tissue fibroblasts and their interactions are crucial for muscle regeneration. Development 138:3625–3637

Nabeshima Y, Hanaoka K, Hayasaka M, Esuml E, Li S, Nonaka I, Nabeshima Y-I (1993) Myogenin gene disruption results in perinatal lethality because of severe muscle defect. Nature 364:532–535

Nguyen-Chi ME, Bryson-Richardson R, Sonntag C, Hall TE, Gibson A, Sztal T, Chua W, Schilling TF, Currie PD (2012) Morphogenesis and cell fate determination within the adaxial cell equivalence group of the zebrafish myotome. PLoS Genet 8(10):e1003014

Ontell M, Feng KC, Klueber K, Dunn RF, Taylor F (1984) Myosatellite cells, growth, and regeneration in murine dystrophic muscle: a quantitative study. Anat Rec 208:159–174

Oppenheim RW (1974) The ontogeny of behaviour in the chick embryo. In: Lehrman DS (ed) Advances in the study of behaviour. Academic, New York, pp 133–172

Ott MO, Bober E, Lyons G, Arnold H, Buckingham M (1991) Early expression of the myogenic regulatory gene, myf-5, in precursor cells of skeletal muscle in the mouse embryo. Development 111(4):1097–1107

Otten C, Abdelilah-Seyfried S (2013) Laser-inflicted injury of Zebrafish embryonic skeletal muscle. J Vis Exp 71

Parichy DM, Elizondo MR, Mills MG, Gordon TN, Engeszer RE (2009) Normal table of postembryonic Zebrafish development: staging by externally visible anatomy of the living fish. Dev Dyn 238:2975–3015

Patterson SE, Mook LB, Devoto SH (2008) Growth in the larval zebrafish pectoral fin and trunk musculature. Dev Dyn 237(2):307–315. doi:10.1002/dvdy.21400

Pirskanen A, Kiefer JC, Hauschka SD (2000) IGFs, insulin, Shh, bFGF, and TGF-beta1 interact synergistically to promote somite myogenesis in vitro. Dev Biol 224(2):189–203

Pownall ME, Gustafsson MK, Emerson CPJ (2002) Myogenic regulatory factors and the specification of muscle progenitors in vertebrate embryos. Annu Rev Cell Dev Biol 18:747–783

Rahimov F, Kunkel LM (2013) Cellular and molecular mechanisms underlying muscular dystrophy. J Cell Biol 201(4):499–510

Rawls A, Valdez MR, Zhang W, Richardson J, Klein WH, Olson EN (1998) Overlapping functions of the myogenic bHLH genes MRF4 and MyoD revealed in double mutant mice. Development 125(13):2349–2358

Relaix F, Rocancourt D, Mansouri A, Buckingham M (2005) A Pax3/Pax7-dependent population of skeletal muscle progenitor cells. Nature 435:948–953

Rescan C, Gauvry L (1996) Genome of the rainbow trout (*Oncorhynchus mykiss*) encodes two distinct muscle regulatory factors with homology to MyoD. Comp Biochem Physiol 113B:711–715

Rescan PY, Montfort J, Fautrel A, Rallière C, Lebret V (2013) Gene expression profiling of the hyperplastic growth zones of the late trout embryo myotome using laser capture microdissection and microarray analysis. BMC Genomics 14(173)

Rome LC, Funke RP, Alexander RM, Lutz G, Aldridge H, Scott F (1988) Why animals have different muscle fibre types. Nature 335:824–827

Roostalu U, Strähle U (2012) In vivo imaging of molecular interactions at damaged sarcolemma. Dev Cell 22:515–529

Ross JJ, Duxson MJ, Harris AJ (1987) Formation of primary and secondary myotubes in rat lumbrical muscles. Development 100:383–394

Rowlerson A, Radaelli G, Mascarello F, Veggetti A (1997) Regeneration of skeletal muscle in two teleost fish: Sparus aurata and Brachydanio rerio. Cell Tissue Res 289(2):311–322

Rowlerson A, Veggetti A (2001) Cellular mechanisms of post-embryonic muscle growth in aquaculture species. In: Johnston IA (ed) Muscle development and growth, vol fish physiology series 18. Academic Press, San Diego, pp 103–140

Rudnicki MA, Jaenisch R (1995) The MyoD family of transcription factors and skeletal myogenesis. Bioessays 17:203–209

Rudnicki MA, Schnegelsberg PN, Stead RH, Braun T, Arnold HH, Jaenisch R (1993) MyoD or Myf-5 is required for the formation of skeletal muscle. Cell 75(7):1351–1359

Saint-Amant L, Drapeau P (1998) Time course of the development of motor behaviors in the zebrafish embryo. J Neurobiol 37(4):622–632

Sander JD, Cade L, Khayter C, Reyon D, Peterson RT, Joung JK, Yeh JR (2011) Targeted gene disruption in somatic zebrafish cells using engineered TALENs. Nat Biotechnol 29:697–698

Sassoon D, Lyons G, Wright WE, Lin V, Lassar A, Weintraub H, Buckingham M (1989) Expression of two myogenic regulatory factors myogenin and MyoD1 during mouse embryogenesis. Nature 341:303–307

Schiaffino S, Reggiani C (2011) Fibre types in mammalian skeletal muscles. Physiol Rev 91:1447–1531

Schienda J, Engleka KA, Jun S, Hansen MS, Epstein JA, Tabin CJ, Kunkel LM, Kardon G (2006) Somitic origin of limb muscle satellite and side population cells. Proc Natl Acad Sci U S A 103:945–950

Schnapp E, Pistocchi AS, Karampetsou E, Foglia E, Lamia CL, Cotelli F, Cossu G (2009) Induced early expression of mrf4 but not myog rescues myogenesis in the myod/myf5 doublemorphant zebrafish embryo. J Cell Sci 122:481–488

Schultz E (1996) Satellite cell proliferative compartments in growing skeletal muscles. Dev Biol 175:84–94

Schultz E, Jaryszak DL (1985) Effects of skeletal muscle regeneration on the proliferation potential of satellite cells. Mech Ageing Dev 30:63–72

Seger C, Hargrave M, Wang X, Chai RJ, Elworthy S, Ingham PW (2011) Analysis of Pax7 expressing myogenic cells in Zebrafish muscle development, injury, and models of disease. Dev Dyn 240(11):2440–2451

Sehnert AJ, Huq A, Weinstein BM, Walker C, Fishman M, Stainier DYR (2002) Cardiac troponin T is essential in sarcomere assembly and cardiac contractility. Nat Genet 31:106–110

Seo HC, Saetre BO, Havik B, Ellingsen S, Fjose A (1998) The zebrafish Pax3 and Pax7 homologues are highly conserved, encode multiple isoforms and show dynamic segment-like expression in the developing brain. Mech Dev 70:49–63

Shi X, Garry DJ (2006) Muscle stem cells in development, regeneration, and disease. Genes Dev 20(13):1692–1708. doi:10.1101/gad.1419406

Sicinski P, Geng Y, Ryder-Cook AS, Barnard EA, Darlison MG, Barnard PJ (1989) The molecular basis of muscular dystrophy in the mdx mouse: a point mutation. Science 244 (4912):1578–1580

Siegel AL, Gurevich DB, Currie PD (2013) A myogenic precursor cell that could contribute to regeneration in zebrafish and its similarity to the satellite cell. FEBS J 280(17):4074–4088

Snow MH (1977) Myogenic cell formation in regenerating rat skeletal muscle injured by mincing. I. A fine structural study. Anat Rec 188:181–200

Steinbacher P, Haslett JR, Obermayer A, Marschallinger J, Bauer HC, Sänger AM, Stoiber W (2007) MyoD and Myogenin expression during myogenic phases in brown trout: a precocious onset of mosaic hyperplasia is a prerequisite for fast somatic growth. Dev Dyn 236(4):1106–1114

Steinbacher P, Marschallinger J, Obermayer A, Neuhofer A, Sanger AM, Stoiber W (2011) Temperature-dependent modification of muscle precursor cell behaviour is an underlying reason for lasting effects on muscle cellularity and body growth of teleost fish. J Exp Biol 214:1791–1801

Stellabotte F, Dobbs-McAuliffe B, Fernandez DA, Feng X, Devoto SH (2007) Dynamic somite cell rearrangements lead to distinct waves of myotome growth. Development 134:1253–1257

Stickland NC (1981) Muscle development in the human fetus as exemplified by m. sartorius: a quantitative study. J Anat 132(4):557–579

Stickney HL, Barresi MJ, Devoto SH (2000) Somite development in Zebrafish. Dev Dyn 219:287–303

Stoiber W, Sänger AM (1996) An electron microscopic investigation into the possible source of new muscle fibres in teleost fish. Anat Embryol (Berl) 194(6):569–579

Szeto DP, Kimelman D (2006) The regulation of mesodermal progenitor cell commitment to somitogenesis subdivides the zebrafish body musculature into distinct domains. Genes Dev 20:1923–1932

Tajbakhsh S, Buckingham ME (2000) The birth of muscle progenitor cells in the mouse: Spatiotemporal considerations. Curr Topic Dev Biol 48:225–268

Telfer WR, Busta AS, Bonnemann CG, Feldman EL, Dowling JJ (2010) Zebrafish models of collagen VI-related myopathies. Hum Mol Genet 19:2433–2444

Turk R, Sterrenburg E, de Meijer EJ, van Ommen GJ, den Dunnen JT, 't Hoen PA (2005) Muscle regeneration in dystrophin-deficient mdx mice studied by gene expression profiling. BMC Genomics 6:98

Valdez MR, Richardson JA, Klein WH, Olson EN (2000) Failure of Myf5 to support myogenic differentiation without myogenin, MyoD, and MRF4. Dev Biol 219(2):287–298

van Raamsdonk W, Pool CW, te Kronnie G (1978) Differentiation of muscle fibre types in the teleost Brachydanio rerio, the zebrafish. Anat Embryol 153:137–155

van Raamsdonk W, te Kronnie G, Pool CW, van de Laarse W (1980) An immonohistochemical and enzymatic characterization of the muscle fibres in myotomal muscle of the teleost *Brachtydanio rerio*. Acta Histochem 67:200–216

Venters SJ, Thorsteinsdottir S, Duxson M (1999) Early development of the myotome in the mouse. Dev Dyn 216(3):219–232

Venuti JM, Morris JH, Vivian JL, Olson EN, Klein WH (1995) Myogenin is required for late but not early aspects of myogenesis during mouse development. J Cell Biol 128(4):563–576

Voytik SL, Przyborski M, Badylak SF, Konieczny SF (1993) Differential expression of muscle regulatory factor genes in normal and denervated adult rat hindlimb muscles. Dev Dyn 198:214–224

Wang YH, Li CK, Lee GH, Tsay HJ, Tsai HJ, Chen YH (2008) Inactivation of zebrafish mrf4 leads to myofibril misalignment and motor axon growth disorganization. Dev Dyn 237:1043–1050

Waterman RE (1969) Development of the lateral musculature in the teleost, *Brachydanio rerio*: a fine structure study. Am J Anat 125:457–493

Weinberg ES, Allende ML, Kelly CS, Abdelhamid A, Murakami T, Andermann P, Doerre OG, Grunwald DJ, Riggleman B (1996) Developmental regulation of zebrafish MyoD in wild-type, no-tail and spadetail embryos. Development 122:271–280

White JD, Scaffidi A, Davies M, McGeachie J, Rudnicki MA, Grounds MD (2000) Myotube formation is delayed but not prevented in MyoD-deficient skeletal muscle: studies in regenerating whole muscle grafts of adult mice. J Histochem Cytochem 48:1531–1544

White RM, Sessa A, Burke C, Bowman T, LeBlanc J, Ceol C, Bourque C, Dovey M, Goessling W, Burns CE, Zon LI (2008) Transparent adult zebrafish as a tool for in vivo transplantation analysis. Cell Stem Cell 2(2):183–189

White RB, Biérinx A-S, Gnocchi VF, Zammit PS (2010) Dynamics of muscle fibre growth during postnatal mouse development. BMC Dev Biol 10:21

Wolff C, Roy S, Ingham PW (2003) Multiple muscle cell identities induced by distinct levels and timing of hedgehog activity in the zebrafish embryo. Curr Biol 13:1169–1181

Yan Z, Choi S, Liu X, Zhang M, Schageman JJ, Lee SY, Hart R, Lin L, Thurmond FA, Williams RS (2003) Highly coordinated gene regulation in mouse skeletal muscle regeneration. J Biol Chem 278:8826–8836

Mechanisms of Myogenic Specification and Patterning

Mordechai Applebaum and Chaya Kalcheim

Abstract Mesodermal somites are initially composed of columnar cells arranged as a pseudostratified epithelium that undergoes sequential and spatially restricted changes to generate the sclerotome and dermomyotome, intermediate structures that develop into vertebrae, striated muscles of the body and limbs, dermis, smooth muscle, and endothelial cells. Regional cues were elucidated that impart differential traits upon the originally multipotent progenitors. How do somite cells and their intermediate progenitors interpret these extrinsic cues and translate them into various levels and/or modalities of intracellular signaling that lead to differential gene expression profiles remains a significant challenge. So is the understanding of how differential fate specification relates to complex cellular migrations prefiguring the formation of body muscles and vertebrae. Research in the past years has largely transited from a descriptive phase in which the lineages of distinct somite-derived progenitors and their cellular movements were traced to a more mechanistic understanding of the local function of genes and regulatory networks underlying lineage segregation and tissue organization. In this chapter, we focus on some major advances addressing the segregation of lineages from the dermomyotome, while discussing both cellular as well as molecular mechanisms, where possible.

1 Introduction

The paraxial mesoderm segments into repetitive epithelial structures termed somites. Subsequently, in response to signals from their environment, epithelial somites dissociate ventrally to give rise to the mesenchymal sclerotome (Scl) and the dorsal epithelial dermomyotome (DM). The Scl generates the vertebral column, ribs, tendons, meninges, and endothelial cells. The DM segregates into the epaxial and hypaxial (body wall and limb) muscles, dermis, endothelial and smooth muscle, and cartilage of the scapula blade, a sublineage that is restricted to specific axial levels (Ben-Yair and Kalcheim 2008; Christ et al. 2004; Scaal and Christ 2004).

M. Applebaum • C. Kalcheim (✉)
Department of Medical Neurobiology, IMRIC and ELSC—Hebrew University—Hadassah Medical School, Jerusalem 9101201, P.O.Box 12272, Israel
e-mail: mordechai.applebaum@mail.huji.ac.il; kalcheim@cc.huji.ac.il

© Springer-Verlag Berlin Heidelberg 2015

B. Brand-Saberi (ed.), *Vertebrate Myogenesis*, Results and Problems in Cell Differentiation 56, DOI 10.1007/978-3-662-44608-9_4

Studies in avian embryos showed that prior to somite dissociation a unique group of progenitors located in the medial wall of the epithelial somite exits the cell cycle, expresses myogenic specification markers such as MyoD and Myf5 and the intermediate filament protein Desmin. These cells, termed “muscle pioneers,” are the first to establish the primary embryonic muscle, the myotome [see Sect. 2 and (Kahane et al. 1998a; Kahane and Kalcheim 1998; Kalcheim et al. 1999)]. Further to the establishment of this initial scaffold, the DM is the epithelium responsible for continuous myotomal growth, first by providing additional postmitotic myocytes and later by providing a reservoir of mitotic progenitors that account for significant myotomal expansion prior to the formation of individual muscles and also for the establishment of muscle satellite cells (see Sect. 3–5). Hence, embryonic muscle formation should be regarded as a progressive process composed of several waves that are well defined in time and space. Growing data from avian embryos, mice, *Xenopus*, and zebrafish models indeed substantiate this general frame of muscle development (Kalcheim et al. 1999; Buckingham and Vincent 2009).

In addition to myotomal cells, the DM also produces a variety of nonstriated muscle phenotypes such as the dorsal dermis that is restricted to the epaxial body domain (Brand Saberi et al. 1996; Ben-Yair et al. 2003), smooth muscle and endothelium lining blood vessels located in the vicinity of their source (Ben-Yair and Kalcheim 2008) and cartilage of the scapula blade, that originates from the hypaxial DM of brachial and thoracic somites in the vicinity of the somatopleura (Huang et al. 2006; Wang et al. 2005). The lineal relations between these phenotypes and myotomal cell types and the gene networks responsible for differential fate acquisition are currently of major interest in the field.

2 The Medial Epithelial Somite Generates the First Wave of Myotomal Myocytes

2.1 The Medial Epithelial Somite Contains Specified Myoblast Progenitors

Pulse-chase experiments with tritiated thymidine complemented by experiments in which short pulses of BrdU were delivered to avian embryos showed that the medial region of the epithelial somite is composed of quiescent progenitors when compared to other somite domains. Later studies confirmed this notion as the cdk inhibitor p27 is intensely expressed in the medial epithelium that faces the neural tube (Halperin-Barlev and Kalcheim 2011). Fate mapping of these early quiescent cells revealed that they generate the first elongated myocytes in the body and were thus termed “myotomal pioneers” (Fig. 1). Furthermore, it was demonstrated that this myogenic source is both earlier and distinct from the mitotically active dorsomedial lip (DML) of the dermomyotome (Kahane et al. 1998a, b, 2002). Being a distinct population of cells, pioneer myoblasts differentially express various genes in the epithelial somite

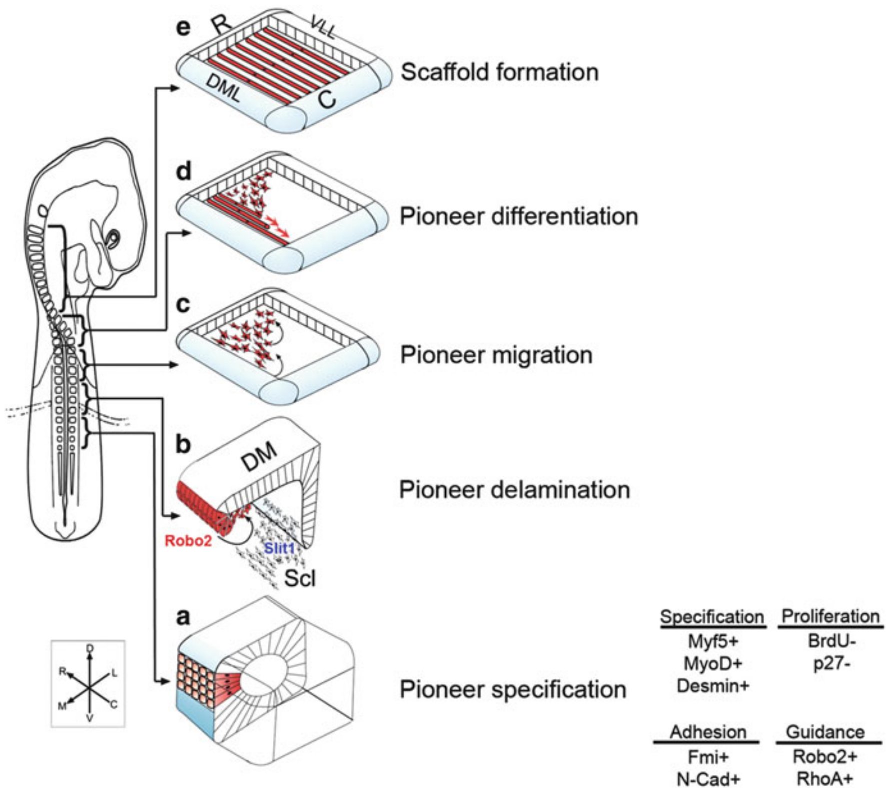


Fig. 1 Pioneer myoblasts stemming from the medial wall of the epithelial somite assemble a scaffold for future myotome development. (**a**) Cells from the medial wall of the epithelial somite are specified to the pioneer lineage. These cells exit the cell cycle (p27-, BrdU-), activate myogenic specification markers (Myf5+, MyoD+, Desmin+), and express adhesion molecules (N-Cad+, Fmi+). (**b**) After delaminating from the epithelial primordium, pioneer myoblasts migrate via guidance molecules (Robo2+ RhoA+) away from Slit1+ sclerotomal cells, thus progressing under the DM in a rostralward direction. (**c**) Next, pioneer cells begin differentiating in both rostro-caudal and medio-lateral directions assembling a stereotypic triangle on the rostro-medial wall of the somite. Cells localized laterally are more immature than their medial peers. (**d**) Medial-most cells differentiate into unit-length myocytes that fully elongate along the rostro-caudal extent of individual segments. This differentiation wave progresses laterally, until a complete scaffold is assembled (**d**). *DM* dermomyotome, *Scl* sclerotome, *DML* dorso-medial lip, *VLL* ventro-lateral lip, *C* caudal, *R* rostral, *L* lateral, *M* medial, *D* dorsal, *V* ventral

and/or during migration and myotome formation (Fig. 1a). For example, the myogenic specification genes *MyoD* and *Myf5* (Kahane et al. 2007) and the intermediate filament protein Desmin (Cinnamon et al. 2006) are already expressed in pioneer progenitors resident in the medial epithelial somite. This pattern is unique to this population as myoblasts originating from the medial DM initiate expression of myogenic genes only after losing their epithelial morphology. Other examples of genes expressed in pioneer myoblasts are the adhesion molecules N-Cadherin and

Fmi (Flamingo). N-Cadherin, which is initially broadly expressed throughout the somite, and thus present in the epithelial pioneers, is downregulated during migration and then upregulated again when they differentiate into myotomal fibers (Cinnamon et al. 2006). *Fmi*, on the other hand, is specifically expressed throughout pioneer myoblast ontogeny (Formstone and Mason 2005). In addition, the guidance molecules *RhoA* and *Robo2* are transcribed in epithelial pioneers prior to dissociation and myocyte formation and were shown to pattern specific aspects of pioneer muscle formation (Halperin-Barlev and Kalcheim 2011).

2.2 A Singular Mode of Pioneer Myoblast Migration and Patterning

Lineage tracing experiments in which the medial epithelial somite was specifically labeled with either the lipophilic dye DiI or with a GFP-encoding plasmid, in which the dynamics of initial desmin immunoreactivity was followed or in which the fate of the earliest tritiated thymidine-negative or BrdU-negative progenitors was traced, showed altogether that, upon somite dissociation, pioneer myoblasts bend underneath the forming dorsomedial lip of the DM, become mesenchymal and engage in a typical directional pattern of migration towards the rostral pole of each somite (Fig. 1b). This process is then followed by a rostral-to-caudal and medial-to-lateral order of fiber differentiation (Kahane et al. 1998a, 2002) (Fig. 1c). Furthermore, at flank regions, the medial (epaxial) pioneer myotome is complemented laterally by a population of early myoblasts emerging from the lateral epithelial somite that initiate myogenesis with a relative delay (Kahane et al. 2007).

As mentioned above, myotome formation by the pioneer cells is the first wave in a multistage process leading to axial muscle development. A second wave arises from the rostral and caudal lips of the epithelial DM and from the dorsomedial and ventrolateral lips (DML and VLL, respectively) (Fig. 2a–b, see Sect. 3). These cells lose the progenitor markers Pax3/7 (Kassar-Duchosoy et al. 2005; Relaix et al. 2005), intercalate among pre-existing pioneer myocytes (Kahane et al. 2002) and become elongated postmitotic myocytes that span the entire length of a segment (Kahane et al. 2002; Gros et al. 2004). The pivotal function of pioneer myoblasts was demonstrated in avian embryos, by using a dominant-negative version of MyoD that prevented their formation in a cell autonomous manner. This severely affected the organization of subsequent waves of myocytes emanating from all the DM lips (Kahane et al. 2007). Thus, pioneer myoblasts serve as a scaffold to pattern the organization of subsequent DM-derived cells that contribute to the developing muscle. Similar interactions between myogenic cells are likely to account for the precision of muscle patterning. In the zebrafish somite, elongation of the fast muscle cells depends on a signal generated by the slow muscle fibers that arise medially adjacent to the notochord and migrate from a medial to a lateral position. This outward relocation causes the fast fibers to elongate in a

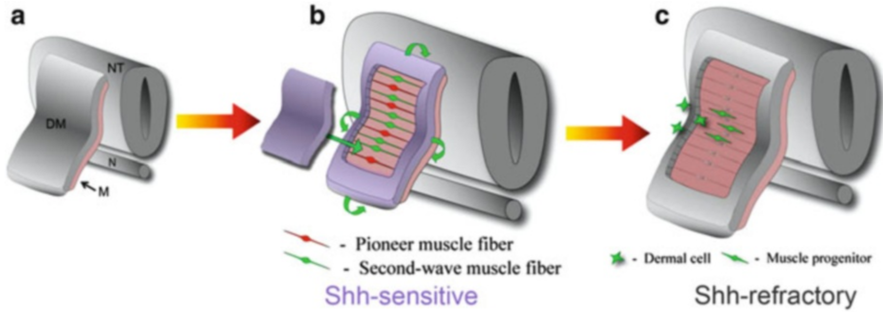


Fig. 2 Two sequential phases of dermomyotome (DM) contribution to myotome. (**a**) The DM is the epithelial remnant of the dorsal somite and covers the pioneer scaffold in the chick embryo (first wave of myogenic cells, see Fig. 1). (**b**) All five domains of the DM, viz., the four lips (DML, VLL, rostral, and caudal) and the early central sheet contribute differentiated myocytes to the developing myotome at E2.5 (second wave of myogenic cells). (**c**) As the DM matures (E3.5) and dissociates its contribution shifts to provide mitotically active muscle progenitors that relocate into the myotome (third wave) and dermal cells that translocate dorsally underneath the ectoderm. While the myogenic phase is Shh-sensitive, the latter phase is Shh-refractory, underpinning the transition between myoblast differentiation and growth. *DM* dermomyotome, *M* myotome, *NT* neural tube, *N* notochord

corresponding medial to lateral gradient (Henry and Amacher 2004). Likewise, in *Drosophila*, founder cells seed the formation of somatic and visceral muscles by associating with fusion-competent myoblasts; in the absence of founder cells, fusion-competent myoblasts remain undifferentiated (Baylies et al. 1998; Bour et al. 2000; Ruiz-Gomez et al. 2000).

Given their significance in patterning myotomal cytoarchitecture, unraveling mechanisms responsible for pioneer myotome development was the next obvious step. First, somite inversions were performed in either rostro-caudal or medio-lateral orientations to examine whether the unique caudo-rostral migration of pioneer myoblasts is somite-intrinsic or, alternatively, depends on environmental cues outside this structure. In both types of operation, grafted pioneers showed an inverted pattern of fiber elongation, demonstrating that they had migrated and differentiated according to their original polarity and were not affected by the surrounding host tissues. Hence, the observed migration of pioneer myoblasts in the caudo-rostral direction prior to differentiation was found to be regulated by somite-intrinsic cues.

In search for the responsible molecules, pioneer myoblasts were found to express the guidance receptor Robo2, while the DM and caudal Scl express its cognate ligand Slit1. Loss of Robo2 or of Scl-derived Slit1 function perturbed both directional cell migration and fiber formation, and their effects were selectively mediated through the Rho GTPase RhoA, but not via RhoB. While Robo2 was shown to act downstream of MyoD/Myf5, its misexpression greatly reduced desmin immunoreactivity but not its transcription. Hence, Slit1-Robo2, via RhoA, acts to pattern formation of the pioneer myotome through regulation of cytoskeletal assembly (Halperin-Barlev and Kalcheim 2011).

3 The Dermomyotome: A Self-Renewing Epithelium That Produces Epaxial and Hypaxial Muscle

The DM is composed of a central epithelial sheet and four contiguous, inwardly curved lips (Fig. 2a). It constitutes a blastema-like zone that generates both primary myocytes that translocate into the underlying myotome and progenitors that remain in the epithelium and account for continuous growth (Ordahl et al. 2001; Kahane et al. 1998b; Gros et al. 2004; Ben-Yair et al. 2003, 2011). Following somite dissociation, the contribution of the DM characterizes the onset of the second wave of myogenesis in avian embryos, which begins with some temporal overlap with respect to the pioneer wave, peaks at E3 and progressively decreases until E4 (Kahane et al. 1998b, 2001; Cinnamon et al. 1999, 2001). Although initially, the dorsomedial (DML) and ventrolateral lips (VLL) of the DM were proposed to be the sole engines driving epaxial and hypaxial myotome growth, respectively (Denetclaw et al. 1997; Denetclaw and Ordahl 2000; Ordahl et al. 2001), subsequent studies established the importance of both rostral and caudal lips of this epithelium in providing elongated myocytes to both domains of the myotome (Kahane et al. 1998b, 2002; Gros et al. 2004; Huang and Christ 2000; Cinnamon et al. 1999, 2006; Denetclaw and Ordahl 2000). More recently, a contribution of the young central DM to myofiber formation was also documented. The above progenitors delaminate into the underlying myotome where they transiently span its entire thickness and keep apico-basal polarity before differentiating into unit-length fibers (Ben-Yair et al. 2011). Altogether, the notion evolves that the entire DM epithelium has the capacity to provide progenitors that contribute to the formation of the early post-mitotic myotomal structure (Nitzan and Kalcheim 2013) (Fig 2b). Moreover, measurements of cell proliferation along the DM, ablation of the DML and direct lineage tracing of DM progenitors showed that the distribution within the myotome of newly added fibers stemming from the DM was equivalent along the entire dorsomedial-to-ventrolateral extent, clearly demonstrating a homogeneous pattern of myotome colonization (Kahane et al. 2002; Ben-Yair et al. 2003).

Notably, postmitotic myocytes are produced as long as the DM persists as an epithelial structure, raising the question of the factor/s responsible for maintaining its epitheliality. The epithelial nature of early somites has been shown to be mediated by canonical, β-catenin-dependent Wnt signaling which activates expression of the bHLH transcription factor Paraxis (Schmidt et al. 2004; Linker et al. 2005), patterns development of the medial DM (Spence et al. 1996; Olivera-Martinez et al. 2001; Capdevila et al. 1998; Ikeya and Takada 1998; Schmidt et al. 2000) and promotes myogenesis (Abu-Elmagd et al. 2010; Munsterberg et al. 1995). Canonical Wnt signaling also maintains epitheliality of the DML (Kruck and Scaal 2012) in agreement with TCF reporter activity in this domain (Brauner et al. 2009; Rios et al. 2010). Surprisingly, the epithelial conformation of the VLL was instead suggested to be regulated by noncanonical, planar cell polarity (PCP)-like Wnt signaling (Kruck and Scaal 2012). Whether the PCP pathway is also instrumental in VLL-derived myogenesis remains to be clarified.

This is of particular interest, as in the medial region, the rostro-caudal orientation of medial fiber elongation was shown to be guided by non-canonical Wnt11-dependent signaling emanating from the DML; *Wnt11* expression in the DML is in turn induced by canonical Wnt signaling from the neural tube (Gros et al. 2009). However, it is unlikely that Wnt11 mediates the directional rostro-caudal elongation of the earlier pioneer myocytes as its transcription is first detected when pioneer differentiation is already underway and, moreover, its expression pattern is homogeneous along the rostro-caudal extent of each segment (Halperin-Barlev and Kalcheim 2011).

Other essential developmental signals, such as Sonic hedgehog (Shh), were classically proposed to induce early myogenesis in the somite and more recently also shown to profoundly affect DM-derived myogenesis in several species (Fig. 2b, see Sect. 6). In addition, BMP signaling from the lateral plate mesoderm was shown to retard myogenesis in the lateral somite by repressing *MyoD* transcription and maintaining cells in a progenitor state (Kahane et al. 2007; Amthor et al. 1999). The next major challenge will be to elucidate the transcriptional networks by which progenitors in different regions of the DM transduce and integrate Shh, Wnt, and BMP signals to modulate the balance between progenitor cell proliferation and differentiation.

Along this line, regulation of DM-derived myogenesis by microRNAs (miRs) is becoming increasingly important as it adds a further level of complexity to our understanding of the spatial and temporal details of the process. miRs act post-transcriptionally to repress gene expression, thus conferring robustness to developmental programs (Stark et al. 2005; Mann et al. 2010). miR-1 and miR-206 were found to be expressed in the DM and myotome of mouse and chick embryos and to repress expression of Pax3 in the DML and VLL of the DM, thus contributing to the stabilization of myoblast commitment and subsequent myogenic differentiation (Goljanek-Whysall et al. 2011).

4 The Central Dermomyotome Sheet: A Source of Muscle and Dermis

4.1 The Epithelial DM Sheet Generates Myocytes While the Dissociating DM Produces Mitotic Muscle Progenitors

The central DM region is initially epithelial but then progressively dissociates while the DML and VLL remain epithelial for about two additional days in the avian embryo. The central DM was classically viewed as a proliferative epithelium that exclusively generates dermis upon dissociation. This view changed during the past years, owing to careful lineage analysis at both the population and single cell levels. When clonally labeled by direct injection of a GFP-encoding plasmid, about 30 %

of single progenitors in the central portion of the nascent DM generated differentiated myocytes 36 h later. These clones were composed of either two fibers each or of a single fiber and no additional cell types were present in the clones, indicating that they stemmed from restricted progenitors in the epithelium and, moreover, that they differentiated following a terminal mitosis or without previous cell division, respectively. In the remaining 70 % of analyzed clones, labeled cells extensively proliferated and were found either in the DM epithelium or in DM-derived progeny. Hence, even at the early stage, the DM is a heterogeneous epithelium, as individual precursors yield either differentiated myocytes or self-renewing cells that account for progressive growth of the structure and for subsequent fates that emerge later when the structure dissociates. This study was the first to show that the early central DM is also myogenic (Ben-Yair et al. 2011). In contrast, if the DM was clonally injected shortly before dissociation most labeled cells that translocated into the myotome remained mesenchymal, mitotically active, and maintained expression of Pax3 and Pax7. The labeled clones also produced dermis as previously described (Fig. 2c) (Ben-Yair and Kalcheim 2005; Gros et al. 2005; Kassar-Duchosoy et al. 2005; Relaix et al. 2005; Kahane et al. 2001). Therefore, the central DM sequentially generates two muscle sublineages: the early, fully epithelial DM sheet is a source of differentiating myocytes, and the late DM, which is about to dissociate, produces mitotic muscle progenitors (Ben-Yair et al. 2011). It is important to mention that these mitotic muscle progenitors are fated to generate muscle fibers and also at least subsets of satellite cells that become apparent later in development (Gros et al. 2005; Kassar-Duchosoy et al. 2005; Relaix et al. 2005). Consistent with these initial findings, it was recently shown that a significant number of adult muscle satellite cells stem from Myf5-positive progenitors whose lineage could be traced back to mouse fetal stages (Biressi et al. 2013). Similarly, murine satellite cells were also shown to emerge from embryonic founder cells that expressed the myogenic regulatory gene MRF4, altogether suggesting a lineage continuity between embryonic progenitors and adult muscle satellite cells (Sambasivan et al. 2013). This notion awaits to be further assessed in avian embryos as well by following, among other possibilities, the lineage of specific subsets of Pax3/7-positive DM progenitors in a stable manner until late fetal or even adult stages. These experiments are now possible thanks to the combination of focal gene electroporation with stable genomic plasmid integration (Sato et al. 2007) and/or by Cre–Lox-dependent stable expression of labeled tags driven by specific enhancers (Nitzan et al. 2013; Avraham et al. 2009).

4.2 Mitotic Orientations Play an Important Role in Fate Segregation of the DM

During development of the nervous system the production of differentiated cell types from epithelial progenitors was shown to depend on controlled orientations of

cell divisions (Kosodo et al. 2004; Huttner and Kosodo 2005; Gotz and Huttner 2005; Zigman et al. 2005; Konno et al. 2008; Morin et al. 2007). Likewise, Ben-Yair et al. (2011) found that maturation of the central DM sheet involves a striking shift in the plane of epithelial cell division from an initial planar orientation, in which the mitotic spindle is oriented parallel to the medio-lateral extent of the DM, into a perpendicular orientation prior to cell dissociation that generates one apically and one basally located daughter cell. This shift in mitotic orientation was shown to depend upon the function of LGN. LGN is the vertebrate homologue of *Drosophila* Partner-of-Insc (Pins), which is essential for spindle positioning by linking the cell cortex with the mitotic spindle (Du et al. 2001; Gotta et al. 2003; Du and Macara 2004; Sanada and Tsai 2005; Siller et al. 2006). LGN-dependent planar cell divisions in the early DM sheet were shown to be required for maintenance of symmetric divisions that allocate progenitors to either DM (self-renewing progenitors) or to the myotome as myocytes. Furthermore, the normal 90° shift in the plane of cell division prior to epithelial dissociation was shown to be essential for generating a balance between muscle versus dermal fates (i.e., asymmetric descendants of a single mitotic event). Hence, LGN-dependent orientation of cell divisions is critical for fate segregation at both stages (Ben-Yair et al. 2011). It remains to be elucidated whether this process involves the asymmetric allocation of cell fate determinants to daughter cells (instructive mechanism) or merely results in the differential translocation of multipotent cells to myotome and dermis where actual specification occurs (permissive mechanism). A concentration of perpendicular mitoses was also reported to predominate in the DML (Venters and Ordahl 2005). The occurrence of such divisions was closely associated with asymmetric localization of the Notch pathway factor Numb, defining such divisions as asymmetric. It is tempting to speculate that in the above perpendicular mitoses the Numb-expressing daughter cells translocate into the myotome whereas the Numb-negative cells remain in the DM epithelium as proliferating precursors. However, no experimental data are currently available to support this notion. Future challenges will be to investigate what are the upstream factors regulating the shift in mitotic orientation and what is the relationship between this process and the epithelial-to-mesenchymal (EMT) of central DM progenitors. Possibly, factors responsible for maintenance of epitheliality would, directly or indirectly, keep cell divisions in a planar orientation (symmetric mitoses). In contrast, lack of such an epithelializing factor/s or emergence of an active EMT-inducing signal would be at least associated with the loss of planar cell divisions and thus be responsible for a shift in mitotic orientation. Understanding of the above processes should then be integrated within a model that also considers their impact on lineage segregation.

5 The Lateral Dermomyotome: A Source of Hypaxial Muscle and of Vascular Fates

5.1 *Clonal Lineage Analysis Reveals the Existence of Bipotent as well as Fate-Restricted Progenitors*

Lineage analysis of the lateral DM performed at the single cell level revealed that of all epithelial domains the lateral region is the most significant source of smooth muscle and endothelium, in addition to producing striated muscle progenitors that settle in myotomes. Notably, progenitors that dissociated from the lateral DM migrated through the ventral sclerotome and adjacent mesenchyme to colonize the closest blood vessels. Typical locations of endothelial and smooth muscle progeny produced by the DM were the cardinal veins, vitelline arteries, and mesonephric, dermal, and somatopleural vessels. Notably, production of endothelial cells was maximal in the lateral epithelial somite (E2) and progressively diminished with development. The proportion of smooth muscle cells was highest both at E2 and E2.5 when compared to E3 when myotomal cells were the major DM derivative (Fig. 3). These results suggest an ordered time course of lineage segregation from the lateral portions of the somite and subsequent DM with an overlap in the time of generation of smooth and striated muscle sublineages. Together, these data indicate that whereas endothelial progenitors are segregated as early as at the epithelial somite stage, smooth and striated muscle sublineages stem from bipotent progenitors still present in the early lateral DM (Ben-Yair and Kalcheim 2008). This finding is generally consistent with results of a retrospective lineage analysis performed in transgenic mice using the nlaacZ reporter, showing the existence of a common progenitor for endothelial, smooth, and striated muscle, likely to be present before somitogenesis (Esner et al. 2006).

Unlike at flank levels of the axis, fate analysis of lateral somite cells performed at hindlimb levels proved that a significant proportion of single progenitors produce both endothelial and striated muscle cells (Kardon et al. 2002a) suggesting that progenitors colonizing the limb are not fate segregated yet. This could be explained by the fact that delaminating lateral progenitors migrate and proliferate extensively on their way into the limb prior to overt differentiation. However, other studies showed that endothelial cells colonize the limb bud first and that this is followed by skeletal muscle cell colonization (He et al. 2003; Tozer et al. 2007; Yvernogeau et al. 2012). Moreover, Yvernogeau et al. (2012) showed that endothelial cells were actually required for myoblast delamination and migration to the limb via yet unknown signals. This study also revealed that cells delaminating from the somites already displayed differential traits, suggesting that also at limb bud levels there is an early fate segregation between endothelial and striated muscle lineages and that if a common progenitor exists its lifespan would be extremely short and restricted to the early mesoderm (Yvernogeau et al. 2012).

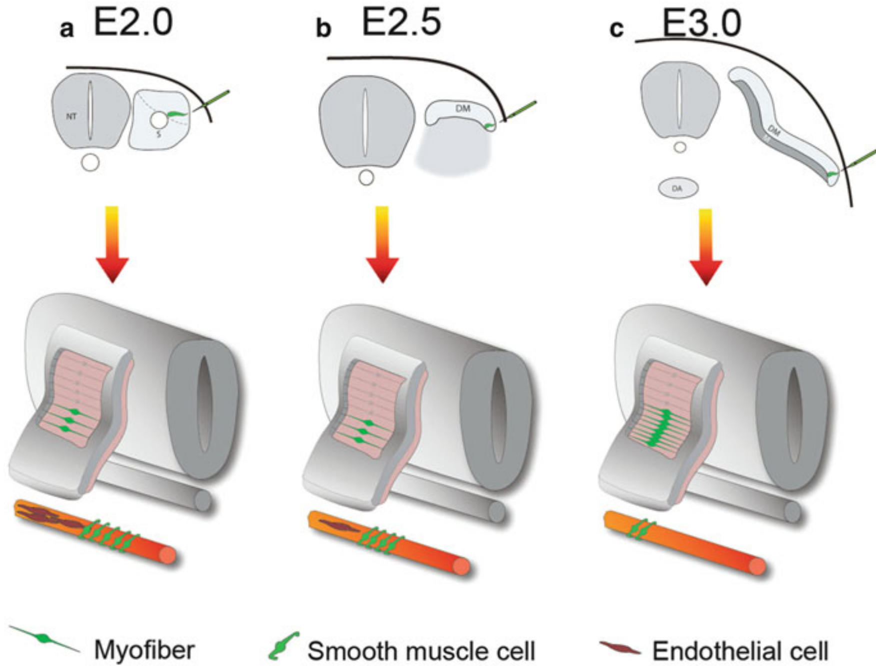


Fig. 3 The lateral aspect of the DM contributes cells to myogenic and vascular fates**.** Single-cell analyses reveal the sequence of lineages issued from the lateral avian DM. (**a**) At E2.0 the lateral DM primarily contributes cells to vascular smooth muscle, with almost equal contribution to endothelium and myofibers of the myotome. (**b**) At E2.5 the smooth muscle fate is still predominant, yet contribution to the endothelium begins to wane with a gradual increase in skeletal myocytes. (**c**) By E3.0 virtually all cells delaminating from the DM colonize the myotome (80 %) while a residual tail of the vascular lineages can still be observed. *NT* neural tube, *S* somite, *DM* dermomyotome, *M* myotome, *DA* dorsal aorta

5.2 Notch Signaling and the Choice Between Myogenic and Vascular Fates

Using gain and loss of function approaches in avian embryos it was demonstrated that Notch activity promotes smooth muscle production while inhibiting striated muscle differentiation from a putative common progenitor. Hence, the choice to become smooth versus striated muscle depends upon Notch signaling (Ben-Yair and Kalcheim 2008).

In line with the transient existence of bipotent smooth-striated muscle progenitors whose definitive fate is influenced by Notch signaling, a recent study revealed the ability of vascular endothelial cells to convert skeletal muscle myoblasts into smooth muscle pericytes (Cappellari et al. 2013). This effect was mediated by PDGF-BB and by the Notch ligand Dll4, both of which led to complete inhibition of

myoblast fusion, retention of proliferative ability, and upregulation of several pericyte markers.

While PDGF-BB alone was ineffective, Dll4 was able to stimulate pericyte cell production, albeit with reduced potency in the absence of PDGF-BB. This effect was specific to the Notch ligand Dll4, as Dll1 or Jagged1 were inactive. Most importantly, Myf5-positive myoblasts derived from later stages (embryonic day 16.5 in mouse embryos) could also be reprogrammed into pericytes using both factors. Finally, the in vivo relevance of Notch signaling was challenged in transgenic mice overexpressing the active intracellular domain of Notch (NICD), resulting in pericyte development at the expense of striated muscle traits.

A further demonstration of the possible switch between smooth and striated muscle phenotypes was provided in a study by Buckingham and colleagues (Lagha et al. 2009). These authors showed that the transcription factor Foxc2 is negatively regulated by Pax3/7 and vice-versa. Compound mutant analyses and manipulation of somite explants indicated that the Pax3/Foxc2 ratio affects myogenic versus vascular cell fate choices, with a higher Pax3/Foxc2 ratio promoting the myogenic lineage and a lower Pax3/Foxc2 ratio promoting the vascular lineages. For example, downregulation of Pax3 leads to specification of undifferentiated somitic cells into smooth muscle. In contrast, in the limb, Pax3 did not appear to commit lateral DM cells to a muscle cell fate (Kardon et al. 2002b). The next step is to unravel the relationship between Notch signaling and these downstream transcription factors in segregating between the various lineages.

In contrast to the lateral DM, progenitors of the DML, “choose” between remaining epithelial or delaminating into the subjacent myotome to generate myocytes. DML cells were shown to transiently activate Notch signaling as a consequence of a temporary interaction with migratory neural crest cells resulting in cells translocating into the myotome and differentiating into myocytes (Rios et al. 2011). The question remains open whether the anti-myogenic activities of Notch previously reported (Vasyutina et al. 2007) versus the pro-myogenic effect observed in the medial DM result from differences in the lateral compared to medial somite context, respectively. Alternatively, and as suggested by Rios and colleagues, short versus long exposure times to a Notch signal were likely to elicit opposite effects in the responding cells.

5.3 BMP Signaling in Vascular Versus Myogenic Differentiation

In the mesoderm, a gradient of *BMP4* mRNA expression is apparent in both the intermediate and lateral plate mesoderm (LPM) where it is intensely transcribed adjacent to the segmental plate, comparatively weaker facing epithelial somites and very faint or undetectable in the LPM juxtaposed to dissociated somites (Sela-Donenfeld and Kalcheim 2002). This rostro-caudal gradient of transcripts suggests

that BMP activity is stronger at early stages and declines progressively. Indeed, at early stages of somitogenesis LPM-derived BMP4 contributes to establishing the medio-lateral polarity of the somite by antagonistically controlling transcription of *Sim1* laterally and *MyoD* medially (Capdevila and Johnson 1998; Pourquie et al. 1996; Tonegawa and Takahashi 1998; Hirsinger et al. 1997). Following this stage, BMP4 was found to prevent premature myogenic differentiation of the lateral somite/DM by inhibiting the onset of *MyoD* transcription in lateral progenitors that already delaminated and localized at their final position in the myotome but, nevertheless, remained in a mesenchymal state (Kahane et al. 2007). Hence, lateral progenitors might halt myogenic differentiation until BMP production is decreased. This might explain why the peak of myogenesis in the lateral DM was found to be delayed when compared to the medial region (Ben-Yair and Kalcheim 2008). As described above (see Sect. 5.1), at early somitic stages, when BMP activity is relatively high, the lateral somite produces endothelial cells. BMP was indeed shown to drive the differentiation of endothelial cells in vitro from embryonic stem cells (Park et al. 2004) and in vivo from the lateral somite (Nimmagadda et al. 2004, 2005). Consistent with these findings, BMP signaling was shown to positively regulate *Vegfr2* expression in the lateral somite and consequent endothelial cell development (Ben-Yair and Kalcheim 2008). Taken together, BMP signaling acts to promote endothelial over striated muscle fates. Since Notch signaling acts similarly to promote development of smooth at the expense of striated muscle lineages from the lateral DM, an interaction between these two signaling systems in this microdomain of the embryo is highly likely and remains to be investigated.

6 The Complexity of Shh Signaling in the DM and in Myogenic Differentiation

6.1 Shh Promotes Myogenic Differentiation of DM Progenitors

Sonic hedgehog (Shh) is a prototypical morphogen that plays essential roles during embryogenesis. Its signal is transduced via two transmembrane proteins, Patched1 (Ptc1) and Smoothened (Smo), and culminates with the regulation of the activity of Gli transcription factors. Gli proteins function either as transcriptional activators or repressors depending on the presence or absence of Shh ligand, respectively (Ribes and Briscoe 2009). Studies focusing on nervous system development reported that the complexity of Shh activity spans several levels of regulation, ranging from posttranscriptional and posttranslational modifications, through secretion from its sources, the notochord and floor plate, transport to long-distances away from the production sites, localized activity at cilia of target cells, and control of activity modulated by networks of transcription factors and cis-regulatory modules

[(Oosterveen et al. 2013; Dessaud et al. 2008; Balaskas et al. 2012) and references therein].

Shh is also active in the somite (Brand-Saberi et al. 1993; Hornik et al. 2004) and during myogenesis where ectopic application caused premature myoblast differentiation (Blagden and Hughes 1999; Du et al. 1997; Borycki et al. 1999; Amthor et al. 1999; Kahane et al. 2001). More detailed studies localized its activity to early somitic cells [(Chiang et al. 1996; Borycki et al. 1999; Gustafsson et al. 2002; Buttitta et al. 2003; McDermott et al. 2005), but see (Teboul et al. 2003)] and also to later DM progenitors (Kahane et al. 2013; Feng et al. 2006; Hammond et al. 2007). Some of the complexity initially uncovered in the nervous system was also found to apply to the myotome. For instance, in zebrafish, different levels and durations of Shh signaling were suggested to specify distinct myotomal cell types (Wolff et al. 2003; Ingham and McMahon 2001; Feng et al. 2006; Maurya et al. 2011; Hammond et al. 2007). In the chick, Shh was found to be necessary for epaxial but not for limb muscle formation (Teillet et al. 1998), yet more recent studies in mouse propose that it also induces the myogenic program in the ventral limb (Hu et al. 2012; Anderson et al. 2012; Krüger et al. 2001). Differential activity of Shh on the epaxial vs. hypaxial myotome at non-limb levels of the axis also became evident from analysis of a mouse reporter line, *Tg(GBS-GFP),* in which GFP expression is induced by the binding of Gli proteins to a concatamer of Gli-binding sites. In these embryos, the GFP signal, that was demonstrated to faithfully reflect Shh activity, was restricted to the epaxial domain. This observation is consistent with the phenotype of Shh mutants in which the hypaxial myotome was largely unaffected (Kahane et al. 2013). In contrast, data from *Xenopus* (Martin et al. 2007), as well as from avian embryos (Kahane et al. 2013), showed that both epaxial as well as hypaxial muscle domains are receptive (Fig. 2b). Notably, even within the epaxial region a heterogeneity in responsiveness to Shh was exposed, likely to stem from differential downstream activities of positive Gli1/2 as opposed to repressor Gli3 activity in different progenitor subsets (Kahane et al. 2013).

How does Shh reach the DM/myotomal area from its source in the notochord? Recent results from murine embryos showed the establishment of a gradient of Shh activity in the sclerotome that tapers off close to the myotome. Furthermore, data from avian embryos, in which sclerotomal Shh was titrated out by misexpressing in these cells hedgehog-interacting protein (Hhip), revealed that its passage through the sclerotome is essential for muscle development (Kahane et al. 2013). However, such a gradient cannot explain the high levels of Shh detected in the myotome itself and therefore additional factors such as *Cdo, Boc, Gas1, Sulfatase1* etc., expressed in DM, in myotome or in both, are likely to modulate locally its activity by mechanisms still to be investigated.

6.2 Shh and Maintenance of the Epithelial DM

An interesting and novel aspect of Shh activity, revealed in the study by Kahane et al. (2013) was the observation that loss of Shh function in avian embryos by treatment with cyclopamine resulted in premature dissociation of the epithelial DM. This phenomenon did not prevent the entry of DM-derived progenitors into the subjacent myotome, but inhibited their differentiation into myofibers and these cells remained mitotically active while keeping *Pax7* expression. Conversely, focal electroporation of Shh to the ectoderm maintained the DM in its epithelial configuration longer than normal and promoted its proliferation. Likewise, an early disorganization of the DM was observed in Shh mutants, followed by cell death, perhaps due to impaired cell proliferation. These findings support the notion that maintenance of the epithelial structure is linked to the ability of the DM to contribute fibers to the underlying myotome rather than mitotic muscle progenitors. This may also be associated with keeping a planar orientation of cell divisions, which characterizes the early phase of myofiber production, when compared to perpendicularly oriented cell divisions that result in production of mitotic myoblasts (see Sect. 4.2). Hence, Shh would be expected to maintain the former orientation of cell divisions, either directly or indirectly. Maintenance of epitheliality and thus of apico-basal cell polarity of the DM could also be essential for keeping cilia in an apical position, which might be necessary for proper transduction of the Shh signal. Of interest is also the relationship between Shh and FGF signaling, which has been shown to act as a myotomal-derived signal to promote DM dissociation (Delfini et al. 2009).

6.3 Loss of Responsiveness to Shh is Associated With the Transition From Muscle Differentiation to Growth

The myogenic effect of Shh on DM progenitors was found to be transient (Fig. 2b–c). Both in mouse as well as in chick embryos, the DM stops responding to a Shh signal close to the time of dissociation, and this is linked to the appearance of many Pax7-positive mitotic myoblasts within the myotome (Kahane et al. 2013). Moreover, lack of responsiveness to Shh was found to be at the level of, or upstream to, Smo signaling, suggesting that the block is very high in the Shh-signaling cascade. This calls for a deeper examination of changes in Shh modulatory proteins acting at the receptor level between the responsive and refractory stages. It also imposes the need for a thorough understanding of how the disorganization of cilia observed upon DM dissociation, from a pure apical to a random localization in the cell surface (Kahane and Kalcheim, unpublished), affects the transduction of a Shh signal in this organelle. Hence, Shh could be one of the responsible signals that maintain the epithelial state of the DM which, in turn, may be necessary for further pro-myogenic responsiveness to this factor.

Another recent study highlighted the significance of Neuregulin1, stemming from neural crest progenitors, that acts through the ErbB3 receptor to regulate muscle development in the mouse. Neuregulin1 maintained the pool of Pax7-positive progenitors while preventing myogenic differentiation (Van Ho et al. 2011). It would be interesting to precisely define the timing of Neuregulin activity, to clarify whether Pax7-positive progenitors in the DM and those resident in muscle similarly respond to this factor (Kalcheim 2011), and whether there is any antagonistic relationship between Neuregulin signaling and Shh or, alternatively, whether Neuregulin acts sequentially following the end of progenitor sensitivity to Shh.

Concluding Remarks

It is becoming increasingly clear that the formation of the somite-derived myotome is far more complex than previously thought. Understanding the logic and details of its ontogeny continues to be essential as a large part of our body muscles transit through a myotomal phase. Moreover, it is clear that proper establishment of this early intermediate structure is pivotal for later establishment of the normal musculature including satellite cells. Current knowledge of the cellular waves responsible for myotome formation sets the grounds for mechanistic studies of which only some were summarized in this chapter and others are yet to be performed. In this context, myotome formation from pioneer myoblasts and from the DM embody the regulation of most relevant processes in development, from cell specification through proliferation, EMT, directional cell migrations, spatial patterning, and terminal differentiation. It is already apparent that the DM is heterogeneous in terms of the state of commitment of its component progenitors at various levels: at different medio-lateral/rostro-caudal regions within the structure itself, at various rostro-caudal domains of the axis and at progressive stages. Likewise, heterogeneity in the early myotomes was also recently documented, both in terms of responsiveness to growth factors as well as in cellular composition. All the above make the paraxial mesoderm a particularly attractive and timely model system to apply state-of-the-art technologies for investigating basic mechanisms of lineage segregation, patterning, and differentiation.

Acknowledgments This work was supported by grants from the Israel Science Foundation (ISF), Association Francaise contre les Myopathies (AFM), and DFG to CK.

References

Abu-Elmagd M, Robson L, Sweetman D, Hadley J, Francis-West P, Munsterberg A (2010) Wnt/Lef1 signaling acts via Pitx2 to regulate somite myogenesis. Dev Biol 337:211–219

Amthor H, Christ B, Patel K (1999) A molecular mechanism enabling continuous embryonic muscle growth—a balance between proliferation and differentiation. Development 126:1041–1053

Anderson C, Williams VC, Moyon B, Daubas P, Tajbakhsh S, Buckingham ME, Shiroishi T, Hughes SM, Borycki AG (2012) Sonic hedgehog acts cell-autonomously on muscle precursor cells to generate limb muscle diversity. Genes Dev 26:2103–2117

Avraham O, Hadas Y, Vald L, Zisman S, Schejter A, Visel A, Klar A (2009) Transcriptional control of axonal guidance and sorting in dorsal interneurons by the Lim-HD proteins Lhx9 and Lhx1. Neural Dev 4:21

Balaskas N, Ribeiro A, Panovska J, Dessaud E, Sasai N, Page KM, Briscoe J, Ribes V (2012) Gene regulatory logic for reading the Sonic Hedgehog signaling gradient in the vertebrate neural tube. Cell 148:273–284

Baylies MK, Bate M, Gomez MR (1998) Myogenesis: a view from *Drosophila*. Cell 93:921–927

Ben-Yair R, Kalcheim C (2005) Lineage analysis of the avian dermomyotome sheet reveals the existence of single cells with both dermal and muscle progenitor fates. Development 132:689–701

Ben-Yair R, Kalcheim C (2008) Notch and bone morphogenetic protein differentially act on dermomyotome cells to generate endothelium, smooth, and striated muscle. J Cell Biol 180:607–618

Ben-Yair R, Kahane N, Kalcheim C (2003) Coherent development of dermomyotome and dermis from the entire mediolateral extent of the dorsal somite. Development 130:4325–4336

Ben-Yair R, Kahane N, Kalcheim C (2011) LGN-dependent orientation of cell divisions in the dermomyotome controls lineage segregation into muscle and dermis. Development 138:4155–4166

Biressi S, Bjornson CR, Carlig PM, Nishijo K, Keller C, Rando TA (2013) Myf5 expression during fetal myogenesis defines the developmental progenitors of adult satellite cells. Dev Biol 379:195–207. doi:10.1016/j.ydbio.2013.04.021

Blagden CS, Hughes SM (1999) Extrinsic influences on limb muscle organisation. Cell Tissue Res 296:141–150

Borycki AG, Brunk B, Tajbakhsh S, Buckingham M, Chiang C, Emerson CP Jr (1999) Sonic hedgehog controls epaxial muscle determination through Myf5 activation. Development 126:4053–4063

Bour BA, Chakravarti M, West JM, Abmayr SM (2000) Drosophila SNS, a member of the immunoglobulin superfamily that is essential for myoblast fusion. Genes Dev 14 (12):1498–1511

Brand Saberi B, Wilting J, Ebensperger C, Christ B (1996) The formation of somite compartments in the avian embryo. Int J Dev Biol 40:411–420

Brand-Saberi B, Ebensperger C, Wilting J, Balling R, Christ B (1993) The ventralizing effect of the notochord on somite differentiation in chick embryos. Anat Embryol 188:239–245

Brauner I, Spicer DB, Krull CE, Venuti JM (2009) Identification of responsive cells in the developing somite supports a role for beta-catenin-dependent Wnt signaling in maintaining the DML myogenic progenitor pool. Dev Dyn 239:222–36. doi:10.1002/dvdy.22098

Buckingham M, Vincent SD (2009) Distinct and dynamic myogenic populations in the vertebrate embryo. Curr Opin Genet Dev 19:444–453

Buttitta L, Mo R, Hui CC, Fan CM (2003) Interplays of Gli2 and Gli3 and their requirement in mediating Shh-dependent sclerotome induction. Development 130:6233–6243

Capdevila J, Johnson RL (1998) Endogenous and ectopic expression of *noggin* suggests a conserved mechanism for regulation of BMP function during limb and somite patterning. Dev Biol 197:205–217

Capdevila J, Tabin C, Johnson RL (1998) Control of dorsoventral somite patterning by Wnt-1 and beta-Catenin. Dev Biol 193:182–194

Cappellari O, Benedetti S, Innocenzi A, Tedesco FS, Moreno-Fortuny A, Ugarte G, Lampugnani MG, Messina G, Cossu G (2013) Dll4 and PDGF-BB convert committed skeletal myoblasts to pericytes without erasing their myogenic memory. Dev Cell 24:586–599

Chiang C, Litingtung Y, Lee E, Young KE, Corden JL, Westphal H, Beachy PA (1996) Cyclopia and defective axial patterning in mice lacking Sonic hedgehog gene function. Nature 383:407–413
Christ B, Huang R, Scaal M (2004) Formation and differentiation of the avian sclerotome. Anat Embryol (Berl) 208(5):333–350
Cinnamon Y, Kahane N, Kalcheim C (1999) Characterization of the early development of specific hypaxial muscles from the ventrolateral myotome. Development 126:4305–4315
Cinnamon Y, Kahane N, Bachelet I, Kalcheim C (2001) The sub-lip domain—a distinct pathway for myotome precursors that demonstrate rostral-caudal migration. Development 128:341–351
Cinnamon Y, Ben-Yair R, Kalcheim C (2006) Differential effects of N-cadherin-mediated adhesion on the development of myotomal waves. Development 133:1101–1112
Delfini MC, De La Celle M, Gros J, Serralbo O, Marics I, Seux M, Scaal M, Marcelle C (2009) The timing of emergence of muscle progenitors is controlled by an FGF/ERK/SNAIL1 pathway. Dev Biol 15:229–237
Denetclaw WF Jr, Ordahl CP (2000) The growth of the dermomyotome and formation of early myotome lineages in thoracolumbar somites of chicken embryos. Development 127:893–905
Denetclaw WF Jr, Christ B, Ordahl CP (1997) Location and growth of epaxial myotome precursor cells. Development 124:1601–1610
Dessaud E, McMahon AP, Briscoe J (2008) Pattern formation in the vertebrate neural tube: a sonic hedgehog morphogen-regulated transcriptional network. Development 135(15):2489–2503
Du Q, Macara IG (2004) Mammalian Pins is a conformational switch that links NuMA to heterotrimeric G proteins. Cell 119(4):503–516
Du SJ, Devoto SH, Westerfield M, Moon RT (1997) Positive and negative regulation of muscle cell identity by members of the hedgehog and TGF-beta gene families. J Cell Biol 139:145–156
Du Q, Stukenberg PT, Macara IG (2001) A mammalian partner of inscuteable binds NuMA and regulates mitotic spindle organization. Nat Cell Biol 3(12):1069–1075
Esner M, Meilhac SM, Relaix F, Nicolas JF, Cossu G, Buckingham ME (2006) Smooth muscle of the dorsal aorta shares a common clonal origin with skeletal muscle of the myotome. Development 133(4):737–749
Feng X, Adiarte EG, Devoto SH (2006) Hedgehog acts directly on the zebrafish dermomyotome to promote myogenic differentiation. Dev Biol 300:736–746
Formstone CJ, Mason I (2005) Expression of the Celsr/flamingo homologue, c-fmi, in the early avian embryo indicates a conserved role in neural tube closure and additional roles in asymmetry and somitogenesis. Dev Dyn 232:409–414
Goljanek-Whysall K, Sweetman D, Abu-Elmagd M, Chapnik E, Dalmay T, Hornstein E, Munsterberg A (2011) MicroRNA regulation of the paired-box transcription factor Pax3 confers robustness to developmental timing of myogenesis. Proc Natl Acad Sci U S A 108
Gotta M, Dong Y, Peterson YK, Lanier SM, Ahringer J (2003) Asymmetrically distributed C. elegans homologs of AGS3/PINS control spindle position in the early embryo. Curr Biol 13(12):1029–1037
Gotz M, Huttner WB (2005) The cell biology of neurogenesis. Nat Rev Mol Cell Biol 6 (10):777–788
Gros J, Scaal M, Marcelle C (2004) A two-step mechanism for myotome formation in chick. Dev Cell 6:875–882
Gros J, Manceau M, Thome V, Marcelle C (2005) A common somitic origin for embryonic muscle progenitors and satellite cells. Nature 435:954–958
Gros J, Serralbo O, Marcelle C (2009) WNT11 acts as a directional cue to organize the elongation of early muscle fibres. Nature 457(7229):589–593
Gustafsson MK, Pan H, Pinney DF, Liu YL, Lewandowski A, Epstein DJ, Emerson CP Jr (2002) *Myf5* is a direct target of long-range Shh signaling and Gli regulation for muscle specification. Genes Dev 16:114–126

Halperin-Barlev O, Kalcheim C (2011) Sclerotome-derived Slit1 drives directional migration and differentiation of Robo2-expressing pioneer myoblasts. Development 138:2935–2945
Hammond CL, Hinits Y, Osborn DP, Minchin JE, Tettamanti G, Hughes SM (2007) Signals and myogenic regulatory factors restrict pax3 and pax7 expression to dermomyotome-like tissue in zebrafish. Dev Biol 302:504–521
He L, Papoutsi M, Huang R, Tomarev SI, Christ B, Kurz H, Wilting J (2003) Three different fates of cells migrating from somites into the limb bud. Anat Embryol (Berl) 207(1):29–34
Henry C, Amacher S (2004) Zebrafish slow muscle cell migration induces a wave of fast muscle morphogenesis. Dev Cell 7:917–923
Hirsinger E, Duprez D, Jouve C, Malapert P, Cooke J, Pourquié O (1997) Noggin acts downstream of Wnt and Sonic Hedgehog to antagonize BMP4 in avian somite patterning. Development 124:4605–4614
Hornik C, Brand-Saberi B, Rudloff S, Christ B, Fuchtbauer EM (2004) Twist is an integrator of SHH, FGF, and BMP signaling. Anat Embryol 209:31–39
Hu JK, McGlinn E, Harfe BD, Kardon G, Tabin CJ (2012) Autonomous and nonautonomous roles of Hedgehog signaling in regulating limb muscle formation. Genes Dev 26:2088–2102
Huang R, Christ B (2000) Origin of the epaxial and hypaxial myotome in avian embryos. Anat Embryol 202:369–374
Huang R, Christ B, Patel K (2006) Regulation of scapula development. Anat Embryol 211(Suppl 1):65–71
Huttner WB, Kosodo Y (2005) Symmetric versus asymmetric cell division during neurogenesis in the developing vertebrate central nervous system. Curr Opin Cell Biol 17(6):648–657
Ikeya M, Takada S (1998) Wnt signaling from the dorsal neural tube is required for the formation of the medial dermomyotome. Development 125:4969–4976
Ingham PW, McMahon AP (2001) Hedgehog signaling in animal development: paradigms and principles. Genes Dev 15:3059–3087
Kahane N, Kalcheim C (1998) Identification of early postmitotic cells in distinct embryonic sites and their possible roles in morphogenesis. Cell Tissue Res 294:297–307
Kahane N, Cinnamon Y, Kalcheim C (1998a) The origin and fate of pioneer myotomal cells in the avian embryo. Mech Dev 74:59–73
Kahane N, Cinnamon Y, Kalcheim C (1998b) The cellular mechanism by which the dermomyotome contributes to the second wave of myotome development. Development 125:4259–4271
Kahane N, Cinnamon Y, Bachelet I, Kalcheim C (2001) The third wave of myotome colonization by mitotically competent progenitors: regulating the balance between differentiation and proliferation during muscle development. Development 128:2187–2198
Kahane N, Cinnamon Y, Kalcheim C (2002) The roles of cell migration and myofiber intercalation in patterning formation of the postmitotic myotome. Development 129:2675–2287
Kahane N, Ben-Yair R, Kalcheim C (2007) Medial pioneer fibers pattern the morphogenesis of early myoblasts derived from the lateral somite. Dev Biol 305:439–450
Kahane N, Ribes V, Kicheva A, Briscoe J, Kalcheim C (2013) The transition from differentiation to growth during dermomyotome-derived myogenesis depends on temporally restricted hedgehog signaling. Development 140:1740–1750
Kalcheim C (2011) Regulation of trunk myogenesis by the neural crest: a new facet of neural crest-somite interactions. Dev Cell 21:187–188
Kalcheim C, Cinnamon Y, Kahane N (1999) Myotome formation: a multistage process. Cell Tissue Res 296:161–173
Kardon G, Campbell JK, Tabin CJ (2002a) Local extrinsic signals determine muscle and endothelial cell fate and patterning in the vertebrate limb. Dev Cell 3(4):533–545
Kardon G, Heanue TA, Tabin CJ (2002b) Pax3 and Dach2 positive regulation in the developing somite. Dev Dyn 224(3):350–355

Kassar-Duchosoy L, Giacone E, Gayraud-Morel B, Jory A, Gomes D, Tajbakhsh S (2005) Pax3/Pax7 mark a novel population of primitive myogenic cells during development. Genes Dev 19:1426–1431

Konno D, Shioi G, Shitamukai A, Mori A, Kiyonari H, Miyata T, Matsuzaki F (2008) Neuroepithelial progenitors undergo LGN-dependent planar divisions to maintain self-renewability during mammalian neurogenesis. Nat Cell Biol 10(1):93–101

Kosodo Y, Roper K, Haubensak W, Marzesco A, Corbeil D, Huttner W (2004) Asymmetric distribution of the apical plasma membrane during neurogenic divisions of mammalian neuroepithelial cells. EMBO J 23(11):2314–2324

Kruck S, Scaal M (2012) Divergent regulation of Wnt-mediated development of the dorsomedial and ventrolateral dermomyotomal lips. Histochem Cell Biol 138:503–514

Krüger M, Mennerich D, Fees S, Schäfer R, Mundlos S, Braun T (2001) Sonic hedgehog is a survival factor for hypaxial muscles during mouse development. Development 128:743–752

Lagha M, Brunelli S, Messina G, Cumano A, Kume T, Relaix F, Buckingham ME (2009) Pax3: Foxc2 reciprocal repression in the somite modulates muscular versus vascular cell fate choice in multipotent progenitors. Dev Cell 17(6):892–899

Linker C, Lesbros C, Gros J, Burrus LW, Rawls A, Marcelle C (2005) beta-Catenin-dependent Wnt signalling controls the epithelial organisation of somites through the activation of paraxis. Development 132(17):3895–3905

Mann M, Barad O, Agami R, Geiger B, Hornstein E (2010) miRNA-based mechanism for the commitment of multipotent progenitors to a single cellular fate. Proc Natl Acad Sci U S A 107:15804–15809

Martin BL, Peyrot SM, Harland RM (2007) Hedgehog signaling regulates the amount of hypaxial muscle development during Xenopus myogenesis. Dev Biol 304:722–734

Maurya AK, Tan H, Souren M, Wang X, Wittbrodt J, Ingham PW (2011) Integration of Hedgehog and BMP signalling by the engrailed2a gene in the zebrafish myotome. Development 138:755–765

McDermott A, Gustafsson M, Elsam T, Hui CC, Emerson CP Jr, Borycki AG (2005) Gli2 and Gli3 have redundant and context-dependent function in skeletal muscle formation. Development 132:345–357

Morin X, Jaouen F, Durbec P (2007) Control of planar divisions by the G-protein regulator LGN maintains progenitors in the chick neuroepithelium. Nat Neurosci 10(11):1440–1448

Munsterberg AE, Kitajewski J, Bumcrot DA, McMahon AP, Lassar AB (1995) Combinatorial signaling by Sonic hedgehog and Wnt family members induces myogenic bHLH gene expression in the somite. Genes Dev 9:2911–2922

Nimmagadda S, Loganathan PG, Wilting J, Christ B, Huang R (2004) Expression pattern of VEGFR-2 (Quek1) during quail development. Anat Embryol (Berl) 208(3):219–224

Nimmagadda S, Geetha Loganathan P, Huang R, Scaal M, Schmidt C, Christ B (2005) BMP4 and noggin control embryonic blood vessel formation by antagonistic regulation of VEGFR-2 (Quek1) expression. Dev Biol 280(1):100–110

Nitzan E, Kalcheim C (2013) Neural crest and somitic mesoderm as paradigms to investigate cell fate decisions during development. Dev Growth Differ 55:60–78

Nitzan E, Krispin S, Pfaltzgraff ER, Klar A, Labosky P, Kalcheim C (2013) A dynamic code of dorsal neural tube genes regulates the segregation between neurogenic and melanogenic neural crest cells. Development 140:2269–2279

Olivera-Martinez I, Thelu J, Teillet M, Dhouailly D (2001) Dorsal dermis development depends on a signal from the dorsal neural tube, which can be substituted by Wnt-1. Mech Dev 100 (2):233–244

Oosterveen T, Kurdija S, Alekseenko Z, Uhde CW, Bergsland M, Sandberg M, Andersson E, Dias JM, Muhr J, Ericson J (2013) Mechanistic differences in the transcriptional interpretation of local and long-range Shh morphogen signaling. Dev Cell 23:1006–1019

Ordahl CP, Berdougo E, Venters SJ, Denetclaw WF Jr (2001) The dermomyotome dorsomedial lip drives growth and morphogenesis of both the primary myotome and dermomyotome epithelium. Development 128:1731–1744

Park C, Afrikanova I, Chung YS, Zhang WJ, Arentson E, Fong Gh G, Rosendahl A, Choi K (2004) A hierarchical order of factors in the generation of FLK1- and SCL-expressing hematopoietic and endothelial progenitors from embryonic stem cells. Development 131(11):2749–2762

Pourquie O, Fan CM, Coltey M, Hirsinger E, Watanabe Y, Breant C, Francis-West P, Brickell P, Tessier-Lavigne M, Le Douarin NM (1996) Lateral and axial signals involved in avian somite patterning: a role for BMP4. Cell 84:461–471

Relaix F, Rocancourt D, Mansouri A, Buckingham M (2005) A Pax3/Pax7-dependent population of skeletal muscle progenitor cells. Nature 435:948–953

Ribes V, Briscoe J (2009) Establishing and interpreting graded Sonic Hedgehog signaling during vertebrate neural tube patterning: the role of negative feedback. Cold Spring Harb Perspect Biol 1:1–16

Rios AC, Denans N, Marcelle C (2010) Real-time observation of Wnt beta-catenin signaling in the chick embryo. Dev Dyn 239:346–353

Rios AC, Serralbo O, Salgado D, Marcelle C (2011) Neural crest regulates myogenesis through the transient activation of NOTCH. Nature 473(7348):532–535

Ruiz-Gomez M, Coutts N, Price A, Taylor MV, Bate M (2000) Drosophila dumbfounded: a myoblast attractant essential for fusion. Cell 102(2):189–198

Sambasivan R, Comai G, Roux IL, Gomes D, Konge J, Dumas G, Cimper C, Tajbakhsh S (2013) Embryonic founders of adult muscle stem cells are primed by the determination gene Mrf4. Dev Biol 381:241–55. doi:10.1016/j.ydbio.2013.04.018

Sanada K, Tsai LH (2005) G protein betagamma subunits and AGS3 control spindle orientation and asymmetric cell fate of cerebral cortical progenitors. Cell 122(1):119–131

Sato Y, Kasai T, Nakagawa S, Tanabe K, Watanabe T, Kawakami K, Takahashi Y (2007) Stable integration and conditional expression of electroporated transgenes in chicken embryos. Dev Biol 305:616–624

Scaal M, Christ B (2004) Formation and differentiation of the avian dermomyotome. Anat Embryol 208:411–424

Schmidt M, Tanaka M, Munsterberg A (2000) Expression of (beta)-catenin in the developing chick myotome is regulated by myogenic signals. Development 127(19):4105–4113

Schmidt C, Stoeckelhuber M, McKinnell I, Putz R, Christ B, Patel K (2004) Wnt 6 regulates the epithelialisation process of the segmental plate mesoderm leading to somite formation. Dev Biol 271:198–209

Sela-Donenfeld D, Kalcheim C (2002) Localized BMP4-noggin interactions generate the dynamic patterning of noggin expression in somites. Dev Biol 246:311–328

Siller KH, Cabernard C, Doe CQ (2006) The NuMA-related Mud protein binds Pins and regulates spindle orientation in Drosophila neuroblasts. Nat Cell Biol 8(6):594–600

Spence MS, Yip J, Erickson CA (1996) The dorsal neural tube organizes the dermamyotome and induces axial myocytes in the avian embryo. Development 122:231–241

Stark A, Brennecke J, Bushati N, Russell RB, Cohen SM (2005) Animal MicroRNAs confer robustness to gene expression and have a significant impact on 3'UTR evolution. Cell 123:1133–1146

Teboul L, Summerbell D, Rigby PW (2003) The initial somitic phase of Myf5 expression requires neither Shh signaling nor Gli regulation. Genes Dev 17:2870–2874

Teillet MA, Lapointe F, Le Douarin NM (1998) The relationships between notochord and floor plate in vertebrate development revisited. Proc Natl Acad Sci USA 95:11733–11738

Tonegawa A, Takahashi Y (1998) Somitogenesis controlled by Noggin. Dev Biol 202:172–182

Tozer S, Bonnin MA, Relaix F, Di Savino S, Garcia-Villalba P, Coumailleau P, Duprez D (2007) Involvement of vessels and PDGFB in muscle splitting during chick limb development. Development 134:2579–2591

Van Ho A, Hayashi S, Brohl D, Aurade F, Rattenbach R, Relaix F (2011) Neural crest cell lineage restricts skeletal muscle progenitor cell differentiation through Neuregulin-ErbB3 signaling. Dev Cell 21:273–287

Vasyutina E, Lenhard DC, Wende H, Erdmann B, Epstein JA, Birchmeier C (2007) RBP-J (Rbpsuh) is essential to maintain muscle progenitor cells and to generate satellite cells. Proc Natl Acad Sci U S A 104(11):4443–4448

Venters SJ, Ordahl CP (2005) Asymmetric cell divisions are concentrated in the dermomyotome dorsomedial lip during epaxial primary myotome morphogenesis. Anat Embryol (Berl) 209 (6):449–460

Wang B, He L, Ehehalt F, Geetha-Loganathan P, Nimmagadda S, Christ B, Scaal M, Huang R (2005) The formation of the avian scapula blade takes place in the hypaxial domain of the somites and requires somatopleure-derived BMP signals. Dev Biol 287(1):11–18

Wolff C, Roy S, PW I (2003) Multiple muscle cell identities induced by distinct levels and timing of hedgehog activity in the zebrafish embryo. Curr Biol 13:1169–1181

Yvernogeau L, Auda-Boucher G, Fontaine-Perus J (2012) Limb bud colonization by somite-derived angioblasts is a crucial step for myoblast emigration. Development 139:277–287

Zigman M, Cayouette M, Charalambous C, Schleiffer A, Hoeller O, Dunican D, McCudden CR, Firnberg N, Barres BA, Siderovski DP, Knoblich JA (2005) Mammalian inscuteable regulates spindle orientation and cell fate in the developing retina. Neuron 48(4):539–545

The Avian Embryo as a Model System for Skeletal Myogenesis

Claire E. Hirst and Christophe Marcelle

Abstract This review will focus on the use of the chicken and quail as model systems to analyze myogenesis and as such will emphasize the experimental approaches that are strongest in these systems—the amenability of the avian embryo to manipulation and in ovo observation. During somite differentiation, a wide spectrum of developmental processes occur such as cellular differentiation, migration, and fusion. Cell lineage studies combined with recent advancements in cell imaging allow these biological phenomena to be readily observed and hypotheses tested extremely rapidly—a strength that is restricted to the avian system. A clear weakness of the chicken in the past has been genetic approaches to modulate gene function. Recent advances in the electroporation of expression vectors, siRNA constructs, and use of tissue specific reporters have opened the door to increasingly sophisticated experiments that address questions of interest not only to the somite/muscle field in particular but also fundamental to biology in general. Importantly, an ever-growing body of evidence indicates that somite differentiation in birds is indistinguishable to that of mammals; therefore, these avian studies complement the complex genetic models of the mouse.

1 Introduction

Somite differentiation represents a wonderfully varied microcosm. In just a few hours, a wide spectrum of developmental processes such as the mesenchymal to epithelial transformation, and the converse epithelial to mesenchymal transition take place along with cellular differentiation, migration, morphogenesis, and fusion. Cell lineage studies combined with recent advancements in cell imaging, such as live cell confocal video microscopy, allow these biological phenomena to be readily observed and hypotheses tested extremely rapidly—a strength that is restricted to the avian system. A clear weakness of the chicken in the past has been

C.E. Hirst (✉) • C. Marcelle (✉)
EMBL Australia, Australian Regenerative Medicine Institute (ARMI), Monash University, Clayton, VIC 3800, Australia
e-mail: claire.hirst@monash.edu; christophe.marcelle@monash.edu

© Springer-Verlag Berlin Heidelberg 2015

B. Brand-Saberi (ed.), *Vertebrate Myogenesis*, Results and Problems in Cell Differentiation 56, DOI 10.1007/978-3-662-44608-9_5

genetic approaches to modulate gene function. Recent advances in electroporation of expression vectors, siRNA constructs, and tissue-specific reporters in defined regions of somites have opened the way to increasingly sophisticated experiments that address questions of interest not only to the somite/muscle field, but also fundamental in biology.

This review will focus on the use of chicken and quail as model systems to analyze myogenesis, and as such will emphasize the experimental approaches that are strongest in this system—the amenability of the avian embryo to manipulation and in ovo observation. Importantly, an ever-growing body of evidence indicates that somite differentiation in birds is identical to that of mammals. As such, avian studies complement the complex genetic models of the mouse. Genetic studies in the mouse (i.e., analyses of transgenic and knockout phenotypes) will be mentioned when they clarify points that have not been answered in the chick.

Given the immense body of literature that covers this field, it is likely that we have not cited everyone; as such we would like to apologize to any of our colleagues that we might have inadvertently overlooked.

2 Somite Differentiation

2.1 Muscle Formation

In amniotes, all skeletal muscle is derived from an embryonic structure known as the somite (Christ and Ordahl 1995), with the exception of the head muscles. Of which the extraocular muscles are derived from the cranial paraxial mesoderm and prechordal mesoderm; the branchial arches are derived from the splanchnic mesoderm and the cranial paraxial mesoderm; while the tongue and laryngeal muscles are derived from the occipital somites. Somites form by the process of segmentation from the presomitic mesoderm under the control of the "segmentation clock." The molecules involved in this process were initially identified in chick embryos in the laboratory of Olivier Pourquié. At the heart of this clock is the oscillatory expression of homologs of the *Drosophila* proteins, hairy (chicken c-hairy, mouse hes1 and hes7) and lunatic fringe (Palmeirim et al. 1997; Jouve et al. 2000; Dale et al. 2003), under the control of NOTCH, FGF, and WNT signaling pathways [for recent reviews see (Wahl et al. 2007; Özbudak and Pourquié 2008; Pourquié 2011; Bénazéraf and Pourquié 2013)]. Complex feedback loop mechanisms of gene products modulating their own expression as well as regulating transcript processing govern the kinetics of segmentation (Lewis 2003; Hoyle and Ish-Horowicz 2013).

These newly formed somites consist of a pseudostratified columnar epithelium surrounding a central cavity, the somitocoel. The ventrolateral portion of the somite undergoes an epithelial to mesenchymal transition (EMT) that results in the formation of the sclerotome. The sclerotome along with mesenchymal cells from the somitocoel gives rise to the axial cartilage, tendons, and bones, while the remaining

epithelial dorsal compartment of the somite is referred to as the dermomyotome. As its name indicates, the dermomyotome gives rise to the dorsal dermis and the primitive skeletal muscle, as well as smooth muscles of the aorta, lymphatic, and vascular endothelial cells (Wilting and Becker 2006; Esner et al. 2006; Pouget et al. 2006; Yusuf and Brand-Saberi 2006).

2.2 Embryonic Origin and Differentiation of Epaxial and Hypaxial Muscles

2.2.1 Morphogenesis

Anatomists have shown that adult muscles can be divided into epaxial and hypaxial muscles. The epaxial muscles are located dorsal to the ribs in the upper trunk region and dorsal to the transverse processes of the vertebrae in the rest of the body, and as such form the back muscles. Hypaxial muscles are found ventrally to the horizontal septum of the vertebrae and form the body wall and abdominal muscles. At the limb level, hypaxial muscles comprise the muscles of the limb and girdle. In amniotes, girdle muscles have become extremely well developed to adapt to terrestrial life, covering a large portion of the trunk epaxial and hypaxial muscles. Regardless of their final location and points of attachment, the epaxial and hypaxial muscles markedly differ in their innervation: the dorsal ramus of the spinal nerve innervates the epaxial muscles, while the ventral ramus innervates the hypaxial muscles.

During embryogenesis, before the future epaxial and hypaxial muscles have formed, the boundary between these two lineages is not obvious. To address the question of their origin, Ordahl performed orthotopic quail–chicken transplants to demonstrate that the epaxial myotome is derived from the medial half of the somite, whereas the hypaxial myotome arises from the lateral half (Ordahl and Le Douarin 1992). One year earlier, Selleck and Stern had used lipophilic fluorescent dyes (DiI and DiO) in the chick embryo to demonstrate that the medial and lateral somite themselves originate from distinct regions of the primitive streak (and Hensen's Node) in the gastrulating embryo (Selleck and Stern 1991). Further refinements and confirmation of these findings came from 3 separate studies. Firstly, the direct labeling of the dorsomedial lip (DML) and ventrolateral lip (VLL) domains with fluorescent dyes in the chick embryo (Denetclaw et al. 1997; Denetclaw and Ordahl 2000); secondly, an elegant technique of retrograde LaacZ labeling in mouse established in JF Nicolas' laboratory (Eloy-Trinquet and Nicolas 2002b; Eloy-Trinquet and Nicolas 2002a), and; thirdly, our own study using electroporation of plasmids coding for fluorescent proteins specifically into the DML and VLL borders of the dermomyotome (Gros et al. 2004). Together, these approaches demonstrate that, in amniotes, the DML and VLL (themselves originating from distinct regions of the primitive streak) give rise exclusively to epaxial and hypaxial muscles, respectively.

These results contrast from those presented in a series of papers by Kalcheim and colleagues, which have extensively characterized a population of cells located in the medial wall of newly formed epithelial somites in chick (somites remain epithelial for 5–7 h before they separate into sclerotome and dermomyotome in the chicken embryo). These cells are identified by the expression of the myogenic regulatory factor (MRF) MyoD and their failure to incorporate BrdU or [^{3}H]-thymidine (Kahane et al. 1998b). Unlike MRF-positive cells in the DML, that rely on axial signals for their induction (see below), MyoD in these cells is induced in a mesoderm-autonomous fashion (Linker et al. 2003). Other properties that distinguish them from DML cells are that they do not have self-renewal capacities (Cinnamon et al. 2006).

Tracing the progeny of these medial wall cells with lipophilic dyes and the exclusion of [^{3}H]-thymidine, these cells were observed to colonize the entire myotome (Kahane et al. 1998b; Cinnamon et al. 1999; Kahane et al. 2002). This cell population, originating from the medial somite, has the unique property of contributing to both epaxial and hypaxial myotome formation. Because of this they were named "pioneer cells" and their translocation into the nascent myotome is referred to as the first wave of myotome colonization. However, most of these experiments were performed in the forelimb region, where the hypaxial myotome is absent due to the emigration of limb and girdle progenitors from the VLL into the limb bud mesenchyme. Therefore the majority of the somitic myotome in the limb region is epaxial as such the identification of cells originating from the medial somitic border along its entire medio-lateral extent is not unexpected. As yet pioneer cells have not been shown to colonize the true hypaxial (i.e. limb or girdle) muscles and therefore it is unclear whether pioneer cells contribute to hypaxial muscles in the adult.

The evolutionary significance of pioneer cells is unclear. In mouse, MyoD is not expressed in newly formed somites (Sassoon et al. 1989). Furthermore, unlike the chick, cells in the medial wall of mouse somite are proliferative (Tam 1981; Rossant and Tam 2002) and in fact do not express any of the MRF. MYF5 is the first of the MRFs to be expressed in mouse and in situ hybridization data (Summerbell et al. 2000) combined with Xgal staining of mouse MYF5 LacZ knock-in embryos (Tajbakhsh et al. 1996) indicates that it is first expressed in the DML as somites separate into sclerotome and dermomyotome, which is similar to the expression of MYF5 in chick embryos at comparable stages of development (Rios et al. 2011). Therefore, while pioneer cells have interesting characteristics, a likely hypothesis is that this population is unique to avians and may not play an important role during myogenesis in mammals.

The DML and the VLL are not the only sources of epaxial and hypaxial muscles, as the anterior and posterior borders of the dermomyotome also contribute to their formation. This was first observed by Kalcheim and colleagues, using lipophilic dye labeling techniques (Kahane et al. 1998a) and was later confirmed by Ordahl's group, using the same technique (Denetclaw and Ordahl 2000). Using the electroporation of a GFP reporter, we extended these studies, by characterizing the relative contribution of the four borders to myotome formation and the precise timing of

myocyte formation at each dermomyotome border (Gros et al. 2004). Altogether these studies demonstrated that all borders of the dermomyotome generate myocytes, but each at its own pace. For instance, the DML generates elongated myocytes approximately 13 h after the somite has formed while the VLL generates myocytes after 27 h. One should note here that all borders ostensibly can generate myocytes as long as they are epithelial. The anterior and posterior borders undergo an EMT (initiated in the center and progressing towards the DML and VLL, see below) a few hours after they have started generating myocytes (our observation). As such their window of opportunity to create myocytes is therefore much shorter than the regenerating epithelia of the DML and VLL, implying that their contribution to the epaxial and hypaxial primary myotome is minor compared to that of the DML and VLL, but this has yet to be experimentally quantified.

In conclusion, during the first stage of muscle morphogenesis, cells arising from the four epithelial borders of the dermomyotome translocate under the dermomyotome to generate differentiated, postmitotic myocytes that organize into the primary myotome. A puzzling but unexplained observation is that, during formation of the primary myotome, the delamination of cells from the borders occurs in an atypical manner. One of the hallmarks of a "classical EMT" [whether in developmental processes or during cancer (Thiery et al. 2009)] is the breakdown of components of the basement membrane and egress of cells through their basal aspect. Here, cells egress through their apical end, in what seems to be a "reverse EMT," a process that deserves to be analyzed in detail.

2.2.2 Genetic Control of Epaxial Muscle Differentiation

While the experiments described above demonstrated that medial and lateral somites contribute to the epaxial and hypaxial muscles respectively, heterotopic graftings of portions of newly formed somites indicated that their fate is not predetermined. Rather, each domain of the somite responds to cues from the environment for its differentiation into muscle (Ordahl and Le Douarin 1992). This important finding paved the way to a large body of research by a multitude of investigators around the world that identified first the tissues, then the molecular cues and pathways regulating the differentiation of somites. The genetic networks underlying the activation of myogenesis (notably the four Myogenic Regulatory Factors, MYF5, MYOD, MYOG, and MRF4 and their relation to the Pax and Six transcription factors) have been exquisitely dissected by a number of laboratories, mainly using genetic approaches in mice. This has provided considerable advances in our knowledge of myogenic differentiation and has been covered in many excellent recent reviews (Bryson-Richardson and Currie 2008; Buckingham and Vincent 2009; Braun and Gautel 2011). Here we will only give a brief overview of the literature relating to the signals regulating epaxial and hypaxial muscle differentiation as well as recently published novel insights into these questions uncovered in the chick model.

The activation of myogenesis in the epaxial domain was found to be regulated by the combinatorial inductive activities of WNT from the dorsal neural tube (and possibly the ectoderm) and SHH from the ventral neural tube and notochord (Stern et al. 1995; Munsterberg et al. 1995). Since this seminal work was published, it has been shown that WNT regulates myogenesis through a β-catenin dependent pathway [the so-called canonical pathway (Borello et al. 2006; Gros et al. 2009)]. Despite numerous publications, the role of SHH remains more obscure, as its effector Gli1-3 displays context-dependent positive and negative regulatory functions on myogenesis, while SHH itself plays either proliferative or instructive function in myogenesis (Teillet et al. 1998; Borycki et al. 1999; McDermott et al. 2005). Furthermore, the uncovering of TCF/LEF and Gli binding sites upstream of the MYF5 locus in mouse indicates that these pathways are direct effectors of MYF5 activation (Borello et al. 2006). An unexpected twist to these findings is a series of studies from George–Weinstein's group, which indicate that mesodermal cells have a tendency to undergo myogenesis in the absence of inducing factors [Gerhart et al. (2007) and references therein] and it is therefore possible that the secreted factors WNT and SHH in fact reinforce a pre-existing bias towards a myogenic path.

Ordahl was the first to show that the DML and VLL act as "cellular growth engines" that not only generate myocytes but also self-renew (Denetclaw and Ordahl 2000; Ordahl et al. 2001). This raises a paradox: although all cells within the DML are presumably equally exposed to factors emanating from axial structures, why do only few at a time undergo myogenic differentiation, while the rest of the population undergoes self-renewal? In most systems in which this has been studied, such binary cell fate decisions are regulated through asymmetric cell division (driven by intrinsic or extrinsic cues), or lateral inhibition mechanisms (Axelrod 2010; Li 2013).

Whilst it is not known whether lateral inhibition plays a role in the DML, the groups of Ordahl and Lassar have described the asymmetric distribution of NUMB protein at the basal side of dividing DML cells. They showed that NUMB is associated with a plane of cell division perpendicular to the apico-basal axis of the cells, which results in a NUMB-positive and a NUMB-negative daughter cell (Venters and Ordahl 2005; Holowacz et al. 2006). Since NUMB belongs to a network of molecules that, during normal development or cancer, regulate asymmetric cell division and fate through its subcellular localization (Knoblich 2010), it was tempting to propose that NUMB regulates the self-renewal versus differentiation fate in the DML. Recent work from the laboratory of Tajbakhsh indicates that NUMB overexpression in the DML of mice leads to a slight increase in both the myogenic and dermomyotomal fates, which does not favor a role of NUMB in the balance between those fates but rather a more general activity on the proliferation of this population (Jory et al. 2009).

A recent study from our group sheds a new light on cell fate decision in the DML. We showed that myogenesis (defined by the expression of MYF5) is initiated in a salt-and-pepper pattern in epithelial cells within the DML (as illustrated in Fig. 1b) and that this activation is dependent on the transient activation of NOTCH

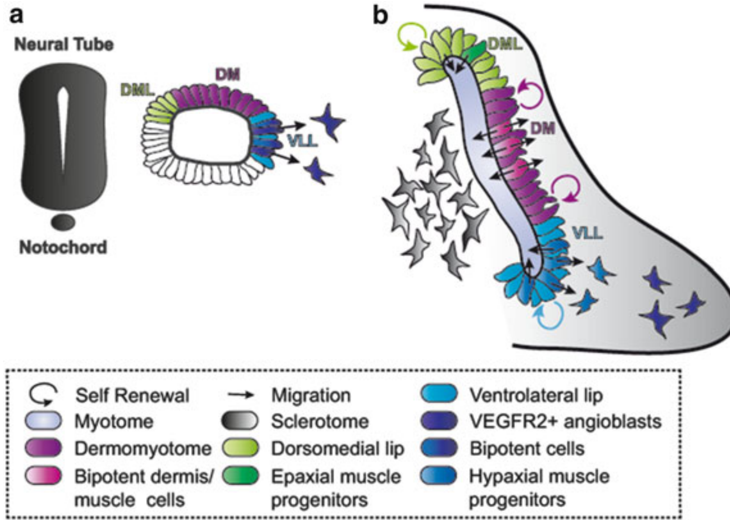


Fig. 1 A model illustrating cell-fate decisions in the somite. (**a**) At the epithelial stage of somite development, molecules from the surrounding axial and lateral structures pattern the presumptive DML, DM, and VLL of the somite. At this stage of somite development VEGFR2 expressing cells are located in the presumptive VLL of the somite, these VEGFR2+ angioblasts migrate into the limb prior to the migration of muscle progenitors from the VLL. (**b**) In all three domains of the somite (DML, DM, and VLL) cells can either self-renew (and contribute to the growth of the region) or commit to differentiation. Activation of cells via NOTCH signaling in the DML, results in Myf5 activation, and translocation in to the myotome. During formation of the primary myotome, the delamination of cells from the epithelium occurs via a "reverse EMT process" by which cells migrate egress through their apical ends (rather than the conventional basal aspect). Following the EMT within the central portion of the DM, cells can either migrate dorsally to contribute to dermis formation or "parachute" into the myotome as muscle progenitor cells. Cells within the VLL initiate differentiation via an as yet unknown signal to translocate into the myotome (to contribute to the growth of the hypaxial myotome in trunk level somites) or migrate into the adjacent limb bud. The VLL also contains bipotential cells that can give rise to either to endothelial cells or muscle progenitor cells

(Rios et al. 2011). The activation or inhibition of NOTCH signaling drives the entire DML population toward a myogenic or self-renewing program, respectively. NOTCH in the DML therefore matches the definition of a binary cell fate choice program. However, its mechanism of action is novel and distinct from the previously identified roles of NOTCH in asymmetric cell division or lateral inhibition mechanisms. Here, the triggering of myogenesis in individual cells relies on the migration of Delta1-expressing neural crest cells across the DML *en route* to their sites of differentiation, a mechanism we named the kiss-and-run mode of signaling (Rios et al. 2011). While, on its own, this mechanism is sufficient to explain the activation of myogenesis observed in the DML, it does not exclude asymmetric cell division or lateral inhibition from playing additional roles in this process. Confocal video-microscopy shows that myogenesis in the DML is a very dynamic process, as cells take as little as 90 min to activate NOTCH and MYF5 and translocate into the

myotome (Rios et al. 2011). Direct observation of this process shows that cells that populate the myotome do not involute in a conveyor belt-like movement around the recurved epithelium of the leading edge of the DML, but rather translocate from a slightly more lateral position in the DML, an observation that had been suggested by Ordahl's group (Denetclaw et al. 2001). Does the finding that NOTCH plays a central role in myogenesis refute previous publications? We do not believe so and unpublished data from our laboratory suggests that NOTCH signaling in fact acts upstream of canonical WNT signaling.

2.2.3 Genetic Control of VLL Differentiation

The signals implicated in the differentiation of the VLL are much less understood. This is in part due to that fact that the VLL is poorly accessible to manipulation and imaging, as it sinks deep into the flank of the embryo during development. A second reason is that the differentiation outcomes are more complex than that of the DML, as with the DML, the cells of the VLL can either differentiate or self-renew. However they give rise to a multitude of additional cell lineages: skeletal muscles, smooth muscles of the aorta, lymphatic vessels, and vascular endothelial cells (Scaal and Christ 2004; Yusuf and Brand-Saberi 2006). How such a bewildering array of differentiation routes is molecularly regulated is unclear at this point. A likely hypothesis is that not all fates are determined at the same time or at the same place.

One of the first signs that the differentiation program is initiated in the hypaxial domain of newly formed somites is the expression of the Vascular Endothelial Growth Factor Receptor 2 (VEGFR2, also named Flk-1/KDR/Quek1). First described in chick and then in mouse, VEGFR2 expression is initiated in the lateral part of epithelial somites as they form (Fig. 1a) and is transiently expressed in a few somite pairs. At later stages of development VEGFR2 expression pattern is restricted to endothelia (Eichmann et al. 1993; Shalaby et al. 1995; Wilting et al. 1997; Ema et al. 2006). As somites were known to be the source of endothelia (Noden 1990; Pardanaud et al. 1996), it was tempting to postulate that its expression within somites identified early endothelial progenitors (i.e., angioblasts). Functional and lineage studies in mouse and chick demonstrated that this is the case (Eichmann et al. 1997; Huber et al. 2004; Yvernogeau et al. 2012), together indicating that angioblasts are specified in the lateral part of newly formed somites. Subsequently, angioblasts migrate out of the VLL to colonize the surrounding mesoderm (Fig. 1b) and form vessels. In the limb region, the migration of angioblasts into the limb bud clearly precedes that of muscle progenitors in both the mouse and chick (Marcelle et al. 2002; Tozer et al. 2007; Yvernogeau et al. 2012). As such, the emigration of angioblasts from the lateral somite takes place long before the VLL has started to generate myocytes.

A careful observation of VEGFR2 expression in chick and mouse shows that it is mosaically expressed in somites (Wilting and Becker 2006; Ema et al. 2006). Clonal analyses in the chick indicates that single cells in somites give rise to

endothelial clones in the limb (Kardon et al. 2002) while in mouse, it was shown that angioblasts leaving somites are committed to the endothelial lineage (Esner et al. 2006; Pouget et al. 2006; Sato et al. 2008; Ohata et al. 2009; Yvernogeau et al. 2012). Altogether, these results strongly suggest that cells expressing VEGFR2 in somites are committed angioblasts. What do the VEGFR2-negative cells within the somites become? The clonal analysis mentioned above showed that skeletal muscle only and mixed clones also derive from somites (Kardon et al. 2002). This suggests that VEGFR2-negative cells are a mix of cells already committed to the skeletal muscle program with cells that are temporally bipotential for the endothelial and muscle lineage (as illustrated in Fig. 1a, b), a finding that was recently confirmed in mouse (Yvernogeau et al. 2012). Combined, these experiments describe an angioblast cell-fate decision within the VLL during the first few hours after somite formation leading to expression of VEGFR2, emigration and further differentiation.

The molecular mechanism of this cell fate decision remains unclear although BMP4 and NOTCH have been implicated. The first factor identified as a key player in the patterning of the hypaxial domain was BMP4 (Pourquié et al. 1996). BMP4 is expressed in the lateral plate mesoderm and in the nascent limb bud and functional analyses showed that it represses myogenesis in the neighboring lateral somite (Pourquié et al. 1996; Linker et al. 2003). More recently, BMP signaling was shown to promote endothelial differentiation (Pouget et al. 2006; Ben-Yair and Kalcheim 2008). However it is not known whether BMP4 acts on the level of VEGFR2 expression within each cell, on the number of cells initiating VEGFR2 expression or on other steps of their differentiation program such as proliferation and survival. NOTCH signaling plays a role in cell fate decisions in many cellular contexts, and thus it is not surprising that it modulates endothelial differentiation. However its exact role is unclear. Kalcheim proposes that NOTCH signaling does not play a role in the initiation of the endothelial fate, but rather in the choice between smooth and skeletal muscle fates (Ben-Yair and Kalcheim 2008). By contrast, Takahashi's group suggests that NOTCH activation is sufficient to direct an endothelial conversion from non-endothelial somitic cells. This work further demonstrated that angioblasts are attracted toward their targets by a receptor/chemokine (CXCR4/SDF1) system (Sato et al. 2008; Ohata et al. 2009). Further analyses will be necessary to determine whether and how BMP and NOTCH signaling cooperate to trigger the endothelial differentiation program in individual cells within somites.

Smooth muscles of the aorta and lymphatic vessels also derive from progenitors in somites. However, compelling data from lineage and genetic studies in chick and mouse strongly suggest that the differentiation of these cell types lies downstream of endothelial differentiation (Wigle and Oliver 1999; Wilting and Becker 2006; Esner et al. 2006; Pouget et al. 2006; Yvernogeau et al. 2012). This suggests that VEGFR2-positive angioblasts that emigrate from somites are in fact pluripotent progenitors. Local cues during their migration or at their sites of differentiation likely define their fate. However, single cell lineage analyses in the chick suggest that smooth muscle and endothelial precursors may have segregated by the time of

somite formation (Ben-Yair and Kalcheim 2008). Here again, further analyses will be needed to clarify this point.

Once the VEGFR2 expressing cells have migrated out of the lateral somite, the situation seems quite similar to that of the DML, as for a number of days, cells within the lateral epithelial border either self renew or generate myocytes (illustrated in Fig. 1b, Denetclaw and Ordahl 2000; Gros et al. 2004). However, little is known about the molecular mechanisms that regulate this binary choice. It is conceivable that the same signaling pathways and signals that were identified in the epaxial domain (WNT, SHH, NOTCH) could be at play, however, the axial structures do not play any significant role in hypaxial muscle differentiation (Rong et al. 1992), and therefore a different source for these cues is required. In mouse, WNT7a in the lateral ectoderm is a likely candidate in the activation of myogenesis in the hypaxial muscle domain (Tajbakhsh et al. 1998), but as it is not expressed in avian ectoderm, it is unclear how universal this finding may be.

The myogenic differentiation that takes place at the anterior and posterior borders of the dermomyotome has not yet been investigated.

2.3 *The Morphogenesis of the Primary Myotome*

2.3.1 Morphogenesis

Using the technique of lipophilic fluorescent dye labeling mentioned above, the groups of Ordahl and Kalcheim pioneered the complex analysis of the primary myotome formation in the chick embryo. They labeled various parts of the somite and later analyzed the progeny of the cells they had labeled. Ordahl observed that cells from the DML and VLL translocate under the dermomyotome where they elongate in the antero-posterior axis of the embryo until reaching the borders of each somite (Denetclaw and Ordahl 2000; Denetclaw et al. 2001). Meanwhile, Kalcheim observed that cells from the medial wall of somite (the pioneer cells) as well as those from the DML and VLL migrate toward the anterior border of the somite before elongating posteriorly (Kahane et al. 1998b; Cinnamon et al. 1999; Cinnamon et al. 2001; Kahane et al. 2002). The advent of in vivo/in ovo electroporation of plasmids coding for fluorescent proteins in somites (Scaal et al. 2004) circumvented some of the limitations of fluorescent dyes. In particular, the exact shape of cells can be easily recognized on fixed samples but also observed under live confocal video-microscopy. Pictures of elongating myocytes expressing GFP at elapsed times in vivo (Gros et al. 2004) and live video-microscopy of this process (Gros et al. 2009) show that cells from the DML translocate under the dermomyotome where they elongate in the antero-posterior axis until reaching the somite borders, thereby confirming the observations of Ordahl and colleagues.

2.3.2 Genetic Control of Myotome Organization

These observations provided the groundwork for a study aimed at understanding the molecular mechanisms regulating the orientation of myocytes parallel to the embryonic axis. This study uncovered that the evolutionary conserved Planar Cell Polarity pathway (PCP) plays a crucial role in this process. Moreover, it provided evidence that WNT11, expressed in the DML, acts as a directional cue to organize the elongation of early muscle fibers (Gros et al. 2009). This elongation phase takes place in a region of the somite that was named transition zone, ventro-lateral to the DML. These results show that the early organogenesis of skeletal muscles in vertebrates is yet another example of an ever growing list of experimental paradigms [from the orientation of cilia in the inner ear to cancer (Wallingford 2012; Luga et al. 2012)] in which PCP plays an important role. Despite this finding, many questions remain to be answered. A crucial point is to understand the mechanism by which the WNT11 signal is translated into polarized growth by elongating myocytes. In *Drosophila*, PCP core proteins are asymmetrically distributed at the cortex of epithelial cells and it is thought that this drives the asymmetric response in these cells (Vladar et al. 2009). We have not yet been able to show that a similar mechanism is at work in elongating myocytes. It is also important to investigate whether PCP is regulating elongation of myocytes at all borders of the somite. Finally it is crucial to determine whether the same mechanisms regulate myotome morphogenesis in mouse, since no obvious PCP phenotype is observed in the trunk muscles of WNT11 knockout mice (Majumdar et al. 2003). WNT5a is expressed in the DML of early somites in chick and the WNT5a knockout mouse displays abnormal myotome development (Yamaguchi et al. 1999). It would be interesting to investigate whether WNT5a in mice plays the same role as WNT11 in chick to regulate myotome organization through the PCP pathway.

2.4 Dermis: Origin, Specification, Morphogenesis

2.4.1 Morphogenesis

The formation of skin appendages like hairs, feathers, and scales depends on the proper differentiation of dermal tissue and its interactions with the overlying epidermis; however, the embryonic development of the dermis is poorly understood. In vertebrates, the dermis originates from three different sources. The craniofacial and cervical dermis is formed by neural crest cells (Le Lièvre and Le Douarin 1975; Couly et al. 1992), the lateral dermis and that of the limbs are derived from the lateral plate mesoderm (Sengel 1971; Olivera-Martinez et al. 2000), while the dorsal dermis arises from regions of the dermomyotome (Olivera-Martinez et al. 2000; Ben-Yair et al. 2003), with some controversy over whether most or only half of the dermomyotome contributes to dorsal dermis. Lineage studies by the group of Kalcheim favor the first hypothesis (Ben-Yair et al. 2003), while work

from Pourquie's group supports the second (Olivera-Martinez et al. 2000). Since the dermomyotome is also the source of resident muscle progenitors (see below), determining whether all or only part of the dermomyotome can give rise to multiple lineages has evident implications in the identification of the molecule(s) that may mediate these fates. Kalcheim's group presents compelling evidence: the labeling of cells with DiI along most of the medio-lateral dermomyotome (excluding the VLL) results in labeled mesenchymal cells positioned beneath the ectoderm 1–2 days later. On the other hand, Pourquie's evidence is just as compelling, showing that the replacement of the lateral half of the chick embryo segmental plate by its quail counterpart indicates no contribution to the dorsal dermis by the end of the experiment. These divergent results could be due to differing experimental protocols, or the reported tendency of quail cells to invade chicken tissues, as suggested by one of the groups, but the reason could be elsewhere. A long-term analysis of the progeny of dorsal dermomyotome electroporated with GFP demonstrated that GFP-labeled mesenchymal cells remained between the ectoderm and the myotome for a prolonged period (Ben-Yair and Kalcheim 2005). However as the incubation time increased, GFP-positive cells derived from the hypaxial domain were found within the myotome, where they expressed markers typical of muscle progenitors (Manceau et al. 2008). Since no obvious sign of cell death was observed, it is likely that cells in the lateral dermomyotome de-epithelialize and are maintained in this state for some time before translocating into the myotome. It is therefore possible that the timing of analysis of the embryos explains the divergent results observed by both groups. As there are no reliable markers to identify the dermis lineage from its emergence in the somite and along its entire differentiation program (see below), this controversy could only be solved by careful long-term lineage analyses of the dermomyotome with appropriate molecular markers.

2.4.2 Genetic Control of Dermis Formation

The molecular mechanisms regulating the development of the dermis are poorly understood. Analyses in mouse and chick showed that the dorsal dermis is composed of two distinct (dorso-medial and dorso-lateral) populations that express, respectively, Msx1 and Dermo-1 (Li et al. 1995; Houzelstein et al. 2000; Scaal et al. 2001). An early marker for the dorso-medial progenitors of dermis is WNT11, which is strongly expressed by mesenchymal cells as they migrate toward the dorsal neural tube. Similarly to WNT11 expression in the DML (Marcelle et al. 1997), WNT11 expression in this cell population is dependent upon WNT1/3a from the dorsal neural tube (Olivera-Martinez et al. 2002) and the removal of the neural tube leads to a defective or absent dorso-medial dermis that can be fully rescued by the implantation of an ectopic dorsal neural tube or WNT1 expressing cells (Olivera-Martinez et al. 2001). Recent work suggests that Wnt11 is required for the specification and/or survival of dermogenic progenitors in the DML and their migration

into the subectodermal space overlying the neural tube (Morosan-Puopolo et al. 2014).

The dorso-lateral dermis arises from an En-1-positive region of the central dermomyotome. En-1 expression relies on survival factors from the notochord (likely SHH) and on unidentified inducing signals from the overlying ectoderm (Olivera-Martinez et al. 2002); however, its expression is independent of WNT11 (C. Marcelle, unpublished). Cells from the En1-positve region de-epithelialize, but do not migrate as extensively as the WNT11-positive dermal progenitors. The EMT of the central dermomyotome can be mimicked by Neurotrophin 3 (NT3) expressed by the neural tube (Brill et al. 1995). However, recent analyses have identified two opposing activities from the ectoderm (WNT6) and primary myotome (FGF) that interact to regulate the timing of the EMT of the dermomyotome. Ectodermal WNT6 maintains the epithelial structure of newly formed somites through a β-catenin-signaling pathway, possible mediated by the b-HLH transcription factor Paraxis (Burgess et al. 1996) as such the EMT of the dermomyotome can be prevented by overexpression of either WNT6 or Paraxis. Conversely, as the somite matures, WNT6 expression in the overlying ectoderm is down regulated—releasing the epithelialization signal (Linker et al. 2005). At the same time, the growing primary myotome delivers increasing amounts of FGF to the overlying dermomyotome which eventually alters the balance, thus triggering the EMT of the dermomyotome through an ERK/Snail1 pathway (Delfini et al. 2009).

While these molecules are likely to regulate dermis formation, their activities do not explain an important observation made by Kalcheim's group—that single cells within the dermomyotome can adopt one of two fates: either differentiate into a dermal progenitor or into a muscle progenitor (illustrated in Fig. 1b, Ben-Yair and Kalcheim 2005). Once again, this is a clear example of a binary cell fate choice and in this case, evidence for a role of asymmetric cell division in this choice is compelling. Kalcheim's group showed that during the growing phase of the dermomyotome, as cells divide symmetrically, their plane of cell division is mostly parallel to the apico-basal axis of epithelial cells (i.e., perpendicular to the plane of the dermomyotome). This results in daughter cells that share similar intracellular components. Focusing on one major player of the adherens junctions in this tissue, N-cadherin, they observed that during EMT the plane of division shifts to become perpendicular to the apico-basal axis and that this results in the asymmetric distribution of N-cadherin in daughter cells. The overexpression or the downregulation of N-cadherin drives the differentiation of dermomyotome cells toward a myogenic or dermis fate, respectively (Cinnamon et al. 2006). In search for an upstream molecular event that regulates spindle orientation, they recently uncovered a crucial role for the G-protein regulator LGN, a known regulator of the orientation of cell division and the differential fate acquisition of *Drosophila* embryonic neuroblasts (Ben-Yair et al. 2011). Since N-cadherin and LGN are ubiquitously expressed throughout the dermomyotome, it is likely that additional cues define the regions/cells that can adopt both fates or not.

2.5 The Origin of Resident Muscle Progenitors and Satellite Cells

2.5.1 Morphogenesis

After the first phase of myotome formation, where the four epithelial borders of the dermomyotome generate the primary myotome, a second phase of muscle growth begins in which the primary myotome is invaded by a population of cells that maintains the capacity to proliferate or to undergo myogenic differentiation, and as such are referred to as the "resident" muscle progenitors (Ben-Yair and Kalcheim 2005; Kassar-Duchossoy et al. 2005; Gros et al. 2005; Relaix et al. 2005). In the chick and mouse, the first visible sign that this second phase is initiated is an EMT in the central portion of the dermomyotome, which occurs in the trunk region in mice at E10.5 and in chick at E3.5. This EMT is characterized by the loss and/or the relocalization of the epithelial markers N-cadherin, β-catenin and F-actin at the adherens junctions located at the apical end of dermomyotomal cells (Gros et al. 2005). The de-epithelialization of the dermomyotome is initiated centrally and progresses in all directions throughout the dermomyotome. As such the EMT at the anterior and posterior borders rapidly follows the EMT of the central dermomyotome. However, the DML and VLL are protected from this wave of de-epithelialization during many days of embryonic development, during which they continue to produce myocytes.

A movie of a dermomyotome electroporated as it undergoes EMT showed that to enter the primary myotome, resident muscle progenitors do not transit around the dermomyotome borders: rather, they directly translocate (they are "parachuted") from the dermomyotome into the myotome. The observation of this process revealed interesting additional features: a cell division preceded the translocation and the plane of division was found to be perpendicular to the apico-basal axis of the mother cell, after which one of the daughter cells entered the myotome, while the other remained in the dermomyotome. This population of resident muscle progenitors is of major importance, since during development they massively contribute to the growth of all embryonic and fetal muscles of the trunk. Moreover, long-term lineage analyses show that satellite cells, the major muscle stem cells of the adult, are derived from this dermomyotomal population (Kassar-Duchossoy et al. 2005; Gros et al. 2005; Relaix et al. 2005).

It has also been reported that the anterior and posterior borders of the dermomyotome can generate muscle progenitors, as recognized by the expression of FGFR4/FREK (Kahane et al. 2001). In chick and mouse, FGFR4 is faintly expressed in the epithelial somites and dermomyotome (Marcelle et al. 1994, 1995; Lagha et al. 2008). In chick, its expression is enhanced at the anterior and posterior borders of the dermomyotome at the time that cells from the still epithelial borders initiate their migration into the primary myotome (Marcelle et al. 2002). Because FGFR4 is specifically expressed by resident muscle progenitors during fetal life (Marcelle et al. 1995), it was tempting to hypothesize that its expression at

the borders reflected the entry of muscle progenitors in the primary myotome. Despite this, we observed that cells from the anterior and posterior epithelial borders electroporated with GFP seemingly all form myocytes (Gros et al. 2004). It is likely that the anterior and posterior borders of the dermomyotome indeed generate muscle progenitors, but only after they have undergone EMT, just as the rest of the de-epithelialized dermomyotome does. To address this question, it would be important to re-examine the fate of anterior and posterior border cells in light of their epithelial or mesenchymal status, but this will require fine-tuning of the experimental protocols and a battery of reliable markers.

2.5.2 Genetic Control of the Emergence and Differentiation of Resident Muscle Progenitors

The emergence of resident muscle progenitors is tightly linked to that of dermal precursors. It is therefore not surprising that the molecular mechanisms that regulate dermis formation (see above) regulate muscle progenitor emergence as well. Indeed, modulating asymmetric cell division in the dermomyotome influences the normal segregation of dermomyotome precursors into myogenic cells (Ben-Yair et al. 2011). However, it is unknown whether factors that regulate the EMT of the dermomyotome also affect the fate of resident muscle progenitors. Genetic analyses in mouse have demonstrated the crucial role that the transcription factors Pax3 and Pax7 cooperatively play in the specification of resident muscle progenitors. In mice deficient for both Pax3 and Pax7, all muscle progenitors are absent and muscle growth is consequently arrested. In those mice, the formation of the primary myotome seems unaffected, but resident muscle progenitors either undergo apoptosis, or assume non-myogenic fates (Relaix et al. 2005).

Once within the muscle masses, it is not known how resident muscle progenitors regulate their cellular choices to either (1) proliferate to maintain or increase the size of their pool, (2) exit the cell cycle to enter the myogenic differentiation program, or (3) become quiescent. The muscle progenitor population comprises a slow-cycling PAX7+/MYF5− stem cells cell population as well as a fast-dividing population of cells that is more clearly engaged in the terminal differentiation program [i.e., expresses both PAX7+/MYF5+ (Picard and Marcelle 2013)]. This heterogeneity in the progenitor pool is also maintained in the adult satellite cell pool (Kuang et al. 2007). This is reminiscent of other stem cell systems where a minor slow cycling stem cell population coexists with a major fast-cycling population of cells with limited differentiation capacities (the so called transit amplifying population). As in other systems where this has been studied, the slow-cycling population display self-renewing capabilities that are important to maintain the long-term regenerating capacity of the muscle (Kuang et al. 2007; Rocheteau et al. 2012; Mascré et al. 2012). The mechanisms that regulate the balance between proliferation and differentiation during embryonic development are poorly understood, however, NOTCH, TGFβ, EGF, and FGF signaling are likely to be important players in this balance between proliferation and myogenic differentiation.

NOTCH and EGF signaling promote the self-renewal of the resident muscle progenitor pool (Schuster-Gossler et al. 2007; Vasyutina et al. 2007; Ho et al. 2011), while the TGF-β family member, Myostatin, plays an opposing role in chick and mouse by promoting myogenic differentiation (Manceau et al. 2008). FGF signaling may also play a similar role in muscle progenitor differentiation, since blocking its signaling with dominant negative FGF receptors or by overexpressing Sprouty leads to a significant decrease in muscle progenitor differentiation (Marics et al. 2002; Lagha et al. 2008). For their obvious interest in cell therapy, most studies have focused on the gene networks regulating the quiescence of satellite cells, or the proliferation, differentiation, and self-renewal of satellite cells in the adult. This has led to the discovery of a growing number of molecules and signaling pathways that may be implicated in these processes. These findings have been largely covered in excellent recent reviews (Fukada et al. 2007; Bentzinger et al. 2012; Cheung et al. 2012; Yin et al. 2013; Montarras et al. 2013). An interesting twist to this ever-growing list of genes and pathways is that there is increasing evidence that resident muscle progenitors and satellite cells constitute a heterogeneous mixture of cell populations. Molecular and cellular differences exist between resident muscle progenitors and satellite cells extracted from different muscle fiber type, muscle origin, and developmental stages (Biressi et al. 2007; Biressi and Rando 2010). The functional significance of those differences is poorly understood, for instance, as the molecular signatures that reflect the environment from which progenitors are isolated are lost when they are transplanted to ectopic locations or placed in culture in vitro (Sambasivan et al. 2009; Harel et al. 2009). It will be important to determine whether the same mechanisms are at work in the adult and in the embryo.

2.5.3 Head and Limb Muscles

While skeletal muscles in the head share a number of features with those of the rest of the body (contractibility, regeneration, fusion, etc.), they also share a number of fundamental differences, the most striking being the gene regulatory networks regulating their early differentiation. A description of the morphogenesis and gene networks involved in their development is outside the scope of this review, however, for further reading please see these recent papers and reviews (Sambasivan et al. 2009; Rios and Marcelle 2009; Harel et al. 2009; Sambasivan et al. 2011). Likewise, the complex molecular networks that regulate the specification, migration, and patterning of the vertebrate limb muscles has been addressed in excellent reviews on the subject (Duprez 2002; Vasyutina and Birchmeier 2006; Towers and Tickle 2009; Butterfield et al. 2010; Murphy and Kardon 2011). .

3 The Future

As recently discussed by Claudio Stern, the developing avian embryo has had a long and illustrious career in developmental biology spanning several millennia of observation and research (Stern 2005). In particular, over the last hundred years, its amenability to manipulation and in ovo observation has allowed the investigation of a diverse range of topics including tissue morphogenesis during gastrulation, embryonic origin of many adult tissues, formation of the circulatory system, as well as the generation of the innate and adaptive immune system. For instance, the pioneering work based on lineage tracing studies using fluorescent lipophilic dyes has permitted short-term analysis of tissue morphogenesis (Stern and Fraser 2001), while chicken–quail transplantation based on the discovery by Nicole Le Douarin that quail nuclei had distinctive nucleoli when stained with Feulgen reagent (Le Douarin and Barq 1969) allowed for long-term (even after hatching) lineage tracing experiments and analysis of cell fate decisions. However despite these elegant anatomical studies, research in the chicken has been hampered for many years by the lack of powerful and easy ways of manipulating gene function during development. This situation has radically changed in the past decade with the advent of in vivo electroporation that permits the efficient transgenesis of specific subpopulations of the chick embryo. This allows us to perform lineage studies to follow the fate and movements of cells during development but also perform gain and loss of function of genes of interest. This can be coupled with the in vivo observation of cell behavior, using classical and two photon confocal microscope technologies (Itasaki et al. 1999; Scaal et al. 2004; Nakamura et al. 2004; Uchikawa 2008; Chuai et al. 2009; Yokota et al. 2011; Rios et al. 2012; Nakamura and Funahashi 2013; Serralbo et al. 2013; Kulesa et al. 2013). The combination of these technologies opens new fields of investigation, until now restricted to more simple systems and makes the chick embryo one of the most exciting and versatile model to characterize in an amniote environment dynamic processes, such as tissue morphogenesis, cell migration and proliferation, signaling, etc. Importantly, as many aspects of embryonic development in birds are indistinguishable from those observed in mouse, this emphasizes the importance of this animal model to understand development in human.

Perhaps no area of embryology is so poorly understood, yet so fascinating, as how tissues and organs are shaped during embryogenesis. *Drosophila* and zebrafish have proven to be powerful systems with which to elucidate the molecular mechanisms of morphogenesis, identifying the signals that pattern the body plan and characterizing cell mechanics and dynamics underling tissue remodeling (Fraser and Harland 2000; Lecuit and Le Goff 2007; Paluch and Heisenberg 2009). The accessibility of the chick embryo to imaging and the development of powerful molecular tools to address gene functions in this organism should prove invaluable assets to address fundamental questions in the amniote embryo.

Acknowledgments We would like to thank Dr Olivier Serralbo and Daniel Sieiro Mosti for their critical reading of the manuscript and Phoebe Kipen for her help with the artwork. The National Health and Medical Research Council (NHMRC) and the Australian Research Council (ARC) support the work in our laboratory. Christophe Marcelle is a Senior Research Fellow of the National Health and Medical Research Council (NHMRC).

References

Axelrod JD (2010) Delivering the lateral inhibition punchline: it's all about the timing. Sci Signal 3:pe38–pe38. doi:10.1126/scisignal.3145pe38

Bénazéraf B, Pourquié O (2013) Formation and segmentation of the vertebrate body axis. Annu Rev Cell Dev Biol 29:1–26. doi:10.1146/annurev-cellbio-101011-155703

Bentzinger CF, Wang YX, Rudnicki MA (2012) Building muscle: molecular regulation of myogenesis. Cold Spring Harb Perspect Biol 4:a008342–a008342. doi:10.1101/cshperspect.a008342

Ben-Yair R, Kalcheim C (2005) Lineage analysis of the avian dermomyotome sheet reveals the existence of single cells with both dermal and muscle progenitor fates. Development 132:689–701

Ben-Yair R, Kalcheim C (2008) Notch and bone morphogenetic protein differentially act on dermomyotome cells to generate endothelium, smooth, and striated muscle. J Cell Biol 180:607–618. doi:10.1083/jcb.200707206

Ben-Yair R, Kahane N, Kalcheim C (2003) Coherent development of dermomyotome and dermis from the entire mediolateral extent of the dorsal somite. Development 130:4325–4336. doi:10.1242/dev.00667

Ben-Yair R, Kahane N, Kalcheim C (2011) LGN-dependent orientation of cell divisions in the dermomyotome controls lineage segregation into muscle and dermis. Development 138:4155–4166. doi:10.1242/dev.065169

Biressi S, Rando TA (2010) Heterogeneity in the muscle satellite cell population. Semin Cell Dev Biol 21:845–854. doi:10.1016/j.semcdb.2010.09.003

Biressi S, Molinaro M, Cossu G (2007) Cellular heterogeneity during vertebrate skeletal muscle development. Dev Biol 308:281–293. doi:10.1016/j.ydbio.2007.06.006

Borello U, Berarducci B, Murphy P et al (2006) The Wnt/beta-catenin pathway regulates Gli-mediated Myf5 expression during somitogenesis. Development 133:3723–3732. doi:10.1242/dev.02517

Borycki AG, Brunk B, Tajbakhsh S et al (1999) Sonic hedgehog controls epaxial muscle determination through Myf5 activation. Development 126:4053–4063

Braun T, Gautel M (2011) Transcriptional mechanisms regulating skeletal muscle differentiation, growth and homeostasis. Nat Rev 12:349–361. doi:10.1038/nrm3118

Brill G, Kahane N, Carmeli C et al (1995) Epithelial-mesenchymal conversion of dermatome progenitors requires neural tube-derived signals: characterization of the role of Neurotrophin-3. Development 121:2583–2594

Bryson-Richardson RJ, Currie PD (2008) The genetics of vertebrate myogenesis. Nat Rev 9:632–646. doi:10.1038/nrg2369

Buckingham M, Vincent SD (2009) Distinct and dynamic myogenic populations in the vertebrate embryo. Curr Opin Genet Dev 19:444–453

Burgess R, Rawls A, Brown D et al (1996) Requirement of the paraxis gene for somite formation and musculoskeletal patterning. Nature 384:570–573. doi:10.1038/384570a0

Butterfield NC, McGlinn E, Wicking C (2010) Chapter nine—The molecular regulation of vertebrate limb patterning. In: Koopman P (ed) Organogenesis in development. Academic, San Diego, CA, pp 319–341

Cheung TH, Quach NL, Charville GW et al (2012) Maintenance of muscle stem-cell quiescence by microRNA-489. Nature 482:524–528. doi:10.1038/nature10834

Christ B, Ordahl CP (1995) Early stages of chick somite development. Anat Embryol 191:381–396

Chuai M, Dormann D, Weijer CJ (2009) Imaging cell signalling and movement in development. Semin Cell Dev Biol 20:947–955. doi:10.1016/j.semcdb.2009.09.001

Cinnamon Y, Kahane N, Kalcheim C (1999) Characterization of the early development of specific hypaxial muscles from the ventrolateral myotome. Development 126:4305–4315

Cinnamon Y, Kahane N, Bachelet I, Kalcheim C (2001) The sub-lip domain–a distinct pathway for myotome precursors that demonstrate rostral-caudal migration. Development 128:341–351

Cinnamon Y, Ben-Yair R, Kalcheim C (2006) Differential effects of N-cadherin-mediated adhesion on the development of myotomal waves. Development 133:1101–1112. doi:10.1242/dev.02291

Couly GF, Coltey PM, Le Douarin NM (1992) The developmental fate of the cephalic mesoderm in quail-chick chimeras. Development 114:1–15

Dale JK, Maroto M, Dequeant ML et al (2003) Periodic notch inhibition by lunatic fringe underlies the chick segmentation clock. Nature 421:275–278. doi:10.1038/nature01244

Delfini M-C, La Celle DM, Gros J et al (2009) The timing of emergence of muscle progenitors is controlled by an FGF/ERK/SNAIL1 pathway. Dev Biol 333:229–237. doi:10.1016/j.ydbio.2009.05.544

Denetclaw WF, Ordahl CP (2000) The growth of the dermomyotome and formation of early myotome lineages in thoracolumbar somites of chicken embryos. Development 127:893–905

Denetclaw WF, Christ B, Ordahl CP (1997) Location and growth of epaxial myotome precursor cells. Development 124:1601–1610

Denetclaw WFJ, Berdougo E, Venters SJ, Ordahl CP (2001) Morphogenetic cell movements in the middle region of the dermomyotome dorsomedial lip associated with patterning and growth of the primary epaxial myotome. Development 128:1745–1755

Duprez D (2002) Signals regulating muscle formation in the limb during embryonic development. Int J Dev Biol 46:915–925

Eichmann A, Marcelle C, Bréant C, Le Douarin NM (1993) Two molecules related to the VEGF receptor are expressed in early endothelial cells during avian embryonic development. Mech Dev 42:33–48

Eichmann A, Corbel C, Nataf V et al (1997) Ligand-dependent development of the endothelial and hemopoietic lineages from embryonic mesodermal cells expressing vascular endothelial growth factor receptor 2. Proc Natl Acad Sci 94:5141–5146

Eloy-Trinquet S, Nicolas J-F (2002a) Cell coherence during production of the presomitic mesoderm and somitogenesis in the mouse embryo. Development 129:3609–3619

Eloy-Trinquet S, Nicolas J-F (2002b) Clonal separation and regionalisation during formation of the medial and lateral myotomes in the mouse embryo. Development 129:111–122

Ema M, Takahashi S, Rossant J (2006) Deletion of the selection cassette, but not cis-acting elements, in targeted Flk1-lacZ allele reveals Flk1 expression in multipotent mesodermal progenitors. Blood 107:111–117. doi:10.1182/blood-2005-05-1970

Esner M, Meilhac SM, Relaix F et al (2006) Smooth muscle of the dorsal aorta shares a common clonal origin with skeletal muscle of the myotome. Development 133:737–749. doi:10.1242/dev.02226

Fraser SE, Harland RM (2000) The molecular metamorphosis of experimental embryology. Cell 100:41–55

Fukada S-I, Uezumi A, Ikemoto M et al (2007) Molecular signature of quiescent satellite cells in adult skeletal muscle. Stem Cells 25:2448–2459. doi:10.1634/stemcells.2007-0019

Gerhart J, Neely C, Elder J et al (2007) Cells that express MyoD mRNA in the epiblast are stably committed to the skeletal muscle lineage. J Cell Biol 178:649–660. doi:10.1083/jcb.200703060

Gros J, Scaal M, Marcelle C (2004) A two-step mechanism for myotome formation in chick. Dev Cell 6:875–882. doi:10.1016/j.devcel.2004.05.006

Gros J, Manceau M, Thomé V, Marcelle C (2005) A common somitic origin for embryonic muscle progenitors and satellite cells. Nature 435:954–958. doi:10.1038/nature03572

Gros J, Serralbo O, Marcelle C (2009) WNT11 acts as a directional cue to organize the elongation of early muscle fibres. Nature 457:589–593. doi:10.1038/nature07564

Harel I, Nathan E, Tirosh-Finkel L et al (2009) Distinct Origins and Genetic Programs of head muscle satellite cells. Dev Cell 16(6):822–832. doi:10.1016/j.devcel.2009.05.007

Ho ATV, Hayashi S, Bröhl D et al (2011) Neural crest cell lineage restricts skeletal muscle progenitor cell differentiation through Neuregulin1-ErbB3 signaling. Dev Cell 21:273–287. doi:10.1016/j.devcel.2011.06.019

Holowacz T, Zeng L, Lassar AB (2006) Asymmetric localization of numb in the chick somite and the influence of myogenic signals. Dev Dyn 235:633–645. doi:10.1002/dvdy.20672

Houzelstein D, Cheraud Y, Auda-Boucher G et al (2000) The expression of the homeobox gene Msx1 reveals two populations of dermal progenitor cells originating from the somites. Development 127:2155–2164

Hoyle NP, Ish-Horowicz D (2013) Transcript processing and export kinetics are rate-limiting steps in expressing vertebrate segmentation clock genes. Proc Natl Acad Sci 110:E4316–E4324. doi:10.1073/pnas.1308811110

Huber TL, Kouskoff V, Joerg Fehling H et al (2004) Haemangioblast commitment is initiated in the primitive streak of the mouse embryo. Nature 432:625–630. doi:10.1038/nature03122

Itasaki N, Bel-Vialar S, Krumlauf R (1999) 'Shocking' developments in chick embryology: electroporation and in ovo gene expression. Nat Cell Biol 1:E203–E207. doi:10.1038/70231

Jory A, Le Roux I, Gayraud-Morel B et al (2009) Numb promotes an increase in skeletal muscle progenitor cells in the embryonic somite. Stem Cells 27:2769–2780. doi:10.1002/stem.220

Jouve C, Palmeirim I, Henrique D et al (2000) Notch signalling is required for cyclic expression of the hairy-like gene HES1 in the presomitic mesoderm. Development 127:1421–1429

Kahane N, Cinnamon Y, Kalcheim C (1998a) The cellular mechanism by which the dermomyotome contributes to the second wave of myotome development. Development 125:4259–4271

Kahane N, Cinnamon Y, Kalcheim C (1998b) The origin and fate of pioneer myotomal cells in the avian embryo. Mech Dev 74:59–73. doi:10.1016/S0925-4773(98)00066-5

Kahane N, Cinnamon Y, Bachelet I, Kalcheim C (2001) The third wave of myotome colonization by mitotically competent progenitors: regulating the balance between differentiation and proliferation during muscle development. Development 128:2187–2198

Kahane N, Cinnamon Y, Kalcheim C (2002) The roles of cell migration and myofiber intercalation in patterning formation of the postmitotic myotome. Development 129:2675–2687

Kardon G, Campbell JK, Tabin CJ (2002) Local extrinsic signals determine muscle and endothelial cell fate and patterning in the vertebrate limb. Dev Cell 3:533–545. doi:10.1016/S1534-5807(02)00291-5

Kassar-Duchossoy L, Giacone E, Gayraud-Morel B et al (2005) Pax3/Pax7 mark a novel population of primitive myogenic cells during development. Gene Dev 19:1426–1431. doi:10.1101/gad.345505

Knoblich JA (2010) Asymmetric cell division: recent developments and their implications for tumour biology. Nat Rev 11:849–860. doi:10.1038/nrm3010

Kuang S, Kuroda K, Le Grand F, Rudnicki MA (2007) Asymmetric self-renewal and commitment of satellite stem cells in muscle. Cell 129:999–1010. doi:10.1016/j.cell.2007.03.044

Kulesa PM, McKinney MC, McLennan R (2013) Developmental imaging: the avian embryo hatches to the challenge. Birth Defects Res C Embryo Today 99:121–133. doi:10.1002/bdrc.21036

Lagha M, Kormish JD, Rocancourt D et al (2008) Pax3 regulation of FGF signaling affects the progression of embryonic progenitor cells into the myogenic program. Genes Dev 22:1828–1837. doi:10.1101/gad.477908

Le Douarin NM, Barq G (1969) Use of Japanese quail as "biological markers" in experimental embryology. C R Acad Sci Hebd Seances Acad Sci D 269:1543–1546

Le Lièvre CS, Le Douarin NM (1975) Mesenchymal derivatives of the neural crest: analysis of chimaeric quail and chick embryos. J Embryol Exp Morphol 34:125–154
Lecuit T, Le Goff L (2007) Orchestrating size and shape during morphogenesis. Nature 450:189–192. doi:10.1038/nature06304
Lewis J (2003) Autoinhibition with transcriptional delay: a simple mechanism for the Zebrafish somitogenesis oscillator. Curr Biol 13:1398–1408
Li R (2013) The art of choreographing asymmetric cell division. Dev Cell 25:439–450. doi:10.1016/j.devcel.2013.05.003
Li L, Cserjesi P, Olson EN (1995) Dermo-1: a novel twist-related bHLH protein expressed in the developing dermis. Dev Biol 172:280–292. doi:10.1006/dbio.1995.0023
Linker C, Lesbros C, Stark MR, Marcelle C (2003) Intrinsic signals regulate the initial steps of myogenesis in vertebrates. Development 130:4797–4807. doi:10.1242/dev.00688
Linker C, Lesbros C, Gros J et al (2005) beta-Catenin-dependent Wnt signalling controls the epithelial organisation of somites through the activation of paraxis. Development 132:3895–3905. doi:10.1242/dev.01961
Luga V, Zhang L, Viloria-Petit AM et al (2012) Exosomes mediate stromal mobilization of autocrine Wnt-PCP signaling in breast cancer cell migration. Cell 151:1542–1556. doi:10.1016/j.cell.2012.11.024
Majumdar A, Vainio S, Kispert A et al (2003) Wnt11 and Ret/Gdnf pathways cooperate in regulating ureteric branching during metanephric kidney development. Development 130:3175–3185. doi:10.1242/dev.00520
Manceau M, Gros J, Savage K et al (2008) Myostatin promotes the terminal differentiation of embryonic muscle progenitors. Genes Dev 22:668–681. doi:10.1101/gad.454408
Marcelle C, Eichmann A, Halevy O et al (1994) Distinct developmental expression of a new avian fibroblast growth factor receptor. Development 120:683–694
Marcelle C, Wolf J, Bronner-Fraser M (1995) The in vivo expression of the FGF receptor FREK mRNA in avian myoblasts suggests a role in muscle growth and differentiation. Dev Biol 172:100–114. doi:10.1006/dbio.1995.0008
Marcelle C, Stark MR, Bronner-Fraser M (1997) Coordinate actions of BMPs, Wnts, Shh and noggin mediate patterning of the dorsal somite. Development 124:3955–3963
Marcelle C, Lesbros C, Linker C (2002) Somite patterning: a few more pieces of the puzzle. Results Probl Cell Differ 38:81–108
Marics I, Padilla F, Guillemot J-F et al (2002) FGFR4 signaling is a necessary step in limb muscle differentiation. Development 129:4559–4569
Mascré G, Dekoninck S, Drogat B et al (2012) Distinct contribution of stem and progenitor cells to epidermal maintenance. Nature 489:257–262. doi:10.1038/nature11393
McDermott A, Gustafsson M, Elsam T et al (2005) Gli2 and Gli3 have redundant and context-dependent function in skeletal muscle formation. Development 132:345–357. doi:10.1242/dev.01537
Montarras D, L'honoré A, Buckingham M (2013) Lying low but ready for action: the quiescent muscle satellite cell. FEBS J 280:4036–4050. doi:10.1111/febs.12372
Morosan-Puopolo G, Balakrishnan-Renuka A, Yusuf F, Chen J, Dai F et al (2014) Wnt11 is required for oriented migration of dermogenic progenitor cells from the dorsomedial lip of the avian dermomyotome. PLoS One 9(3):e92679. doi:10.1371/journal.pone.0092679
Munsterberg AE, Kitajewski J, Bumcrot DA et al (1995) Combinatorial signaling by Sonic hedgehog and Wnt family members induces myogenic bHLH gene expression in the somite. Genes Dev 9:2911–2922
Murphy M, Kardon G (2011) Chapter one—Origin of vertebrate limb muscle: the role of progenitor and myoblast populations. In: Pavlath GK (ed) Curr Top Dev Biol. 1–32
Nakamura H, Funahashi J (2013) Electroporation: past, present and future. Dev Growth Differ 55:15–19. doi:10.1111/dgd.12012

Nakamura H, Katahira T, Sato T et al (2004) Gain- and loss-of-function in chick embryos by electroporation. The Chick in Developmental Biology 121:1137–1143. doi:10.1016/j.mod.2004.05.013
Noden DM (1990) Origins and assembly of avian embryonic blood vessels. Ann N Y Acad Sci 588:236–249. doi:10.1111/j.1749-6632.1990.tb13214.x
Ohata E, Tadokoro R, Sato Y et al (2009) Notch signal is sufficient to direct an endothelial conversion from non-endothelial somitic cells conveyed to the aortic region by CXCR4. Dev Biol 335:33–42. doi:10.1016/j.ydbio.2009.08.010
Olivera-Martinez I, Coltey M, Dhouailly D, Pourquie O (2000) Mediolateral somitic origin of ribs and dermis determined by quail-chick chimeras. Development 127:4611–4617
Olivera-Martinez I, Thélu J, Teillet M-A, Dhouailly D (2001) Dorsal dermis development depends on a signal from the dorsal neural tube, which can be substituted by Wnt-1. Mech Dev 100:233–244
Olivera-Martinez I, Missier S, Fraboulet S et al (2002) Differential regulation of the chick dorsal thoracic dermal progenitors from the medial dermomyotome. Development 129:4763–4772
Ordahl CP, Le Douarin NM (1992) Two myogenic lineages within the developing somite. Development 114:339–353
Ordahl CP, Berdougo E, Venters SJ, Denetclaw WFJ (2001) The dermomyotome dorsomedial lip drives growth and morphogenesis of both the primary myotome and dermomyotome epithelium. Development 128:1731–1744
Özbudak EM, Pourquié O (2008) The vertebrate segmentation clock: the tip of the iceberg. Curr Opin Genet Dev 18:317–323. doi:10.1016/j.gde.2008.06.007
Palmeirim I, Henrique D, Ish-Horowicz D, Pourquie O (1997) Avian hairy gene expression identifies a molecular clock linked to vertebrate segmentation and somitogenesis. Cell 91:639–648
Paluch E, Heisenberg C-P (2009) Biology and physics of cell shape changes in development. Curr Biol 19:R790–R799. doi:10.1016/j.cub.2009.07.029
Pardanaud L, Luton D, Prigent M et al (1996) Two distinct endothelial lineages in ontogeny, one of them related to hemopoiesis. Development 122:1363–1371
Picard CA, Marcelle C (2013) Two distinct muscle progenitor populations coexist throughout amniote development. Dev Biol 373:141–148
Pouget C, Gautier R, Teillet M-A, Jaffredo T (2006) Somite-derived cells replace ventral aortic hemangioblasts and provide aortic smooth muscle cells of the trunk. Development 133:1013–1022. doi:10.1242/dev.02269
Pourquié O (2011) Vertebrate segmentation: from cyclic gene networks to scoliosis. Cell 145:650–663. doi:10.1016/j.cell.2011.05.011
Pourquié O, Fan C-M, Coltey M et al (1996) Lateral and axial signals involved in avian somite patterning: a role for BMP4. Cell 84:461–471. doi:10.1016/S0092-8674(00)81291-X
Relaix F, Rocancourt D, Mansouri A, Buckingham M (2005) A Pax3/Pax7-dependent population of skeletal muscle progenitor cells. Nature 435:948–953
Rios AC, Marcelle C (2009) Head muscles: aliens who came in from the cold? Dev Cell 16:779–780. doi:10.1016/j.devcel.2009.06.004
Rios AC, Serralbo O, Salgado D, Marcelle C (2011) Neural crest regulates myogenesis through the transient activation of NOTCH. Nature 473:532–535. doi:10.1038/nature09970
Rios AC, Marcelle C, Serralbo O (2012) Gene loss-of-function and live imaging in chick embryos. Methods Mol Biol 839:105–117. doi:10.1007/978-1-61779-510-7_9
Rocheteau P, Gayraud-Morel B, Siegl-Cachedenier I et al (2012) A subpopulation of adult skeletal muscle stem cells retains all template DNA strands after cell division. Cell 148:112–125. doi:10.1016/j.cell.2011.11.049
Rong PM, Teillet MA, Ziller C, Le Douarin NM (1992) The neural tube/notochord complex is necessary for vertebral but not limb and body wall striated muscle differentiation. Development 115:657–672

Rossant J, Tam PPL (2002) Mouse development. In: Patterning, morphogenesis, and organogenesis. Academic, San Diego, pp xiii–xv
Sambasivan R, Gayraud-Morel B, Dumas G et al (2009) Distinct regulatory cascades govern extraocular and branchiomeric muscle progenitor cell fates. Dev Cell 16:810–821
Sambasivan R, Kuratani S, Tajbakhsh S (2011) An eye on the head: the development and evolution of craniofacial muscles. Development 138:2401–2415. doi:10.1242/dev.040972
Sassoon D, Lyons G, Wright WE et al (1989) Expression of two myogenic regulatory factors myogenin and MyoDl during mouse embryogenesis. Nature 341:303–307. doi:10.1038/341303a0
Sato Y, Watanabe T, Saito D et al (2008) Notch mediates the segmental specification of angioblasts in somites and their directed migration toward the dorsal aorta in avian embryos. Dev Cell 14:890–901
Scaal M, Christ B (2004) Formation and differentiation of the avian dermomyotome. Anat Embryol 208:411–424. doi:10.1007/s00429-004-0417-y
Scaal M, Füchtbauer E-M, Brand-Saberi B (2001) cDermo-1 expression indicates a role in avian skin development. Anat Embryol 203(1):1–7. doi:10.1007/PL00008244
Scaal M, Gros J, Lesbros C, Marcelle C (2004) In ovo electroporation of avian somites. Dev Dyn 229:643–650. doi:10.1002/dvdy.10433
Schuster-Gossler K, Cordes R, Gossler A (2007) Premature myogenic differentiation and depletion of progenitor cells cause severe muscle hypotrophy in Delta1 mutants. Proc Natl Acad Sci U S A 104:537–542. doi:10.1073/pnas.0608281104
Selleck MA, Stern CD (1991) Fate mapping and cell lineage analysis of Hensen's node in the chick embryo. Development 112:615–626
Sengel P (1971) The organogenesis and arrangement of cutaneous appendages in birds. Adv Morphog 9:181–230
Serralbo O, Picard CA, Marcelle C (2013) Long term, inducible gene loss-of-function in the chicken embryo. Genesis 51(3):372–380. doi:10.1002/dvg.22388
Shalaby F, Rossant J, Yamaguchi TP et al (1995) Failure of blood-island formation and vasculogenesis in Flk-1-deficient mice. Nature 376:62–66. doi:10.1038/376062a0
Stern CD (2005) The chick: a great model system becomes even greater. Dev Cell 8:9–17. doi:10.1016/j.devcel.2004.11.018
Stern CD, Fraser SE (2001) Tracing the lineage of tracing cell lineages. Nat Cell Biol 3:E216–E218. doi:10.1038/ncb0901-e216
Stern HM, Brown AM, Hauschka SD (1995) Myogenesis in paraxial mesoderm: preferential induction by dorsal neural tube and by cells expressing Wnt-1. Development 121:3675–3686
Summerbell D, Ashby PR, Coutelle O et al (2000) The expression of Myf5 in the developing mouse embryo is controlled by discrete and dispersed enhancers specific for particular populations of skeletal muscle precursors. Development 127:3745–3757
Tajbakhsh S, Bober E, Babinet C et al (1996) Gene targeting the myf-5 locus with nlacZ reveals expression of this myogenic factor in mature skeletal muscle fibres as well as early embryonic muscle. Dev Dyn 206:291–300
Tajbakhsh S, Borello U, Vivarelli E et al (1998) Differential activation of Myf5 and MyoD by different Wnts in explants of mouse paraxial mesoderm and the later activation of myogenesis in the absence of Myf5. Development 125:4155–4162
Tam P (1981) The control of somitogenesis in mouse embryos. J Embryol Exp Morphol 65:103–128
Teillet M, Watanabe Y, Jeffs P et al (1998) Sonic hedgehog is required for survival of both myogenic and chondrogenic somitic lineages. Development 125:2019–2030
Thiery JP, Acloque H, Huang RYJ, Nieto MA (2009) Epithelial-mesenchymal transitions in development and disease. Cell 139:871–890. doi:10.1016/j.cell.2009.11.007
Towers M, Tickle C (2009) Growing models of vertebrate limb development. Development 136:179–190. doi:10.1242/dev.024158

Tozer S, Bonnin M-A, Relaix F et al (2007) Involvement of vessels and PDGFB in muscle splitting during chick limb development. Development 134:2579–2591. doi:10.1242/dev.02867

Uchikawa M (2008) Enhancer analysis by chicken embryo electroporation with aid of genome comparison. Dev Growth Differ 50:467–474. doi:10.1111/j.1440-169X.2008.01028.x

Vasyutina E, Birchmeier C (2006) The development of migrating muscle precursor cells. Anat Embryol 211(Suppl 1):37–41. doi:10.1007/s00429-006-0118-9

Vasyutina E, Lenhard DC, Wende H et al (2007) RBP-J (Rbpsuh) is essential to maintain muscle progenitor cells and to generate satellite cells. Proc Natl Acad Sci U S A 104:4443–4448. doi:10.1073/pnas.0610647104

Venters SJ, Ordahl CP (2005) Asymmetric cell divisions are concentrated in the dermomyotome dorsomedial lip during epaxial primary myotome morphogenesis. Anat Embryol 209:449–460. doi:10.1007/s00429-005-0461-2

Vladar EK, Antic D, Axelrod JD (2009) Planar cell polarity signaling: the developing cell's compass. Cold Spring Harb Perspect Biol 1:a002964–a002964. doi:10.1101/cshperspect.a002964

Wahl MB, Deng C, Lewandoski M, Pourquié O (2007) FGF signaling acts upstream of the NOTCH and WNT signaling pathways to control segmentation clock oscillations in mouse somitogenesis. Development 134:4033–4041. doi:10.1242/dev.009167

Wallingford JB (2012) Planar cell polarity and the developmental control of cell behavior in vertebrate embryos. Annu Rev Cell Dev Biol 28:627–653. doi:10.1146/annurev-cellbio-092910-154208

Wigle JT, Oliver G (1999) Prox1 function is required for the development of the murine lymphatic system. Cell 98:769–778

Wilting J, Becker J (2006) Two endothelial cell lines derived from the somite. Anat Embryol 211:57–63. doi:10.1007/s00429-006-0120-2

Wilting J, Eichmann A, Christ B (1997) Expression of the avian VEGF receptor homologues Quek1 and Quek2 in blood-vascular and lymphatic endothelial and non-endothelial cells during quail embryonic development. Cell Tissue Res 288:207–223. doi:10.1007/s004410050807

Yamaguchi TP, Bradley A, McMahon AP, Jones S (1999) A Wnt5a pathway underlies outgrowth of multiple structures in the vertebrate embryo. Development 126:1211–1223

Yin H, Price F, Rudnicki MA (2013) Satellite cells and the muscle stem cell niche. Physiol Rev 93:23–67. doi:10.1152/physrev.00043.2011

Yokota Y, Saito D, Tadokoro R, Takahashi Y (2011) Genomically integrated transgenes are stably and conditionally expressed in neural crest cell-specific lineages. Dev Biol 353:382–395

Yusuf F, Brand-Saberi B (2006) The eventful somite: patterning, fate determination and cell division in the somite. Anat Embryol 211:21–30. doi:10.1007/s00429-006-0119-8

Yvernogeau L, Auda-Boucher G, Fontaine-Perus J (2012) Limb bud colonization by somite-derived angioblasts is a crucial step for myoblast emigration. Development 139:277–287. doi:10.1242/dev.067678

Head Muscle Development

Eldad Tzahor

Abstract The developmental paths that lead to the formation of skeletal muscles in the head are distinct from those operating in the trunk. Craniofacial muscles are associated with head and neck structures. In the embryo, these structures derive from distinct mesoderm populations. Distinct genetic programs regulate different groups of muscles within the head to generate diverse muscle specifications. Developmental and lineage studies in vertebrates and invertebrates demonstrated an overlap in progenitor populations derived from the pharyngeal mesoderm that contribute to certain head muscles and the heart. These studies reveal that the genetic program controlling pharyngeal muscles overlaps with that of the heart. Indeed cardiac and craniofacial birth defects are often linked. Recent studies suggest that early chordates, the last common ancestor of tunicates and vertebrates, had an ancestral pharyngeal mesoderm lineage that later during evolution gave rise to both heart and craniofacial structures. This chapter summarizes studies related to the origins, signaling, genetics, and evolution of the head musculature, highlighting its heterogeneous characteristics in all these aspects.

1 Skeletal Muscle Formation

Myogenesis, the formation of muscle tissue, takes place during embryonic development, postnatal growth, and regeneration. Vertebrate movement depends on skeletal muscles in our body, which are derived from the segmented structures in the developing embryo composed of paraxial mesoderm cells, known as somites (Christ and Ordahl 1995). Myogenesis begins with the commitment of mesoderm precursor cells to the myogenic lineage. This is followed by proliferation of myoblasts and their differentiation into postmitotic myocytes that fuse to form multinucleated myotubes. Skeletal muscles utilize distinct regulatory networks upstream of a core myogenic program driven by myogenic regulatory factors (MRFs) to initiate myogenesis at different anatomical locations (e.g., head and

E. Tzahor (✉)
Department of Biological Regulation, Weizmann Institute of Science, Rehovot 76100, Israel
e-mail: eldad.tzahor@weizmann.ac.il

© Springer-Verlag Berlin Heidelberg 2015

B. Brand-Saberi (ed.), *Vertebrate Myogenesis*, Results and Problems in Cell Differentiation 56, DOI 10.1007/978-3-662-44608-9_6

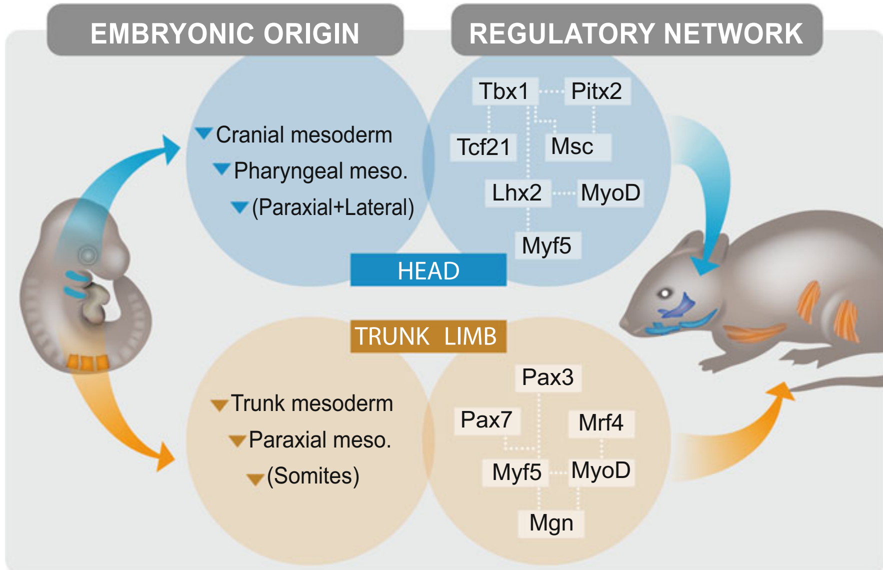


Fig. 1 Distinct mesodermal origins and regulatory networks promote muscle formation in the trunk and head regions. Trunk myogenesis is derived from the paraxial mesoderm, embryonic structures that are known as somites (*orange*). Head muscles are derived from several mesoderm populations located anterior to the somites (*blue*). These mesoderm cells are termed pharyngeal mesoderm and prechordal mesoderm. Pharyngeal mesoderm cells (composed of both paraxial and lateral regions of the cranial mesoderm) populate the pharyngeal arches and are the progenitors of distinct pharyngeal muscles. Embryological and genetic studies indicate that distinct regulatory networks control the formation of head and trunk skeletal muscles

trunk) (Bryson-Richardson and Currie 2008; Buckingham and Vincent 2009) (Fig. 1). Because many transcription factors that regulate the fate of muscle progenitors have been identified, skeletal muscle tissue constitutes an ideal model for the study of organogenesis and regeneration (Berkes and Tapscott 2005; Tajbakhsh 2005).

Molecular and technical advances in the last two decades have yielded a detailed understanding of the embryology of this tissue, and its genetic regulation by these transcription factors, including the paired/homeobox genes *Pax3* and *Pax7*, and the myogenic regulatory factors (MRFs) *Myf5*, *MyoD*, *Mrf4*, and *Myogenin* (Buckingham 2001; Kassar-Duchossoy et al. 2004) (Fig. 1). Skeletal muscle was the first tissue in which a determination gene for cell fate, *MyoD*, was identified in vertebrates (Weintraub et al. 1991). MRFs are crucial for myogenic specification and determination, as shown by genetic loss-of-function analyses. Specifically, knockout studies in mice have shown that MyoD, Myf5, and Mrf4 function as myogenic determination factors; in the absence of all three, skeletal muscle fails to form. Myogenin acts as a differentiation factor, as are Mrf4 and MyoD, promoting the differentiation of myoblasts into myotubes.

Questions related to the inductive processes and the molecular events underpinning embryonic myogenesis are currently under intensive study worldwide. Answers to these questions may provide basic insights into muscle biology, as well as to the growing field of regenerative medicine as myogenesis in adult muscle stem cells recapitulates that of the embryo.

2 Head Muscles

In contrast to our understanding of how skeletal muscle is formed in the trunk, less is known about the tissues and molecules that induce the formation of the head musculature. It is clear that the developmental paths that lead to the formation of skeletal muscles in the head are distinct from those operating in the trunk (Fig. 1). This chapter summarizes studies of the origins, composition, signaling, genetics, and evolution of distinct craniofacial muscles.

Approximately 60 muscles exist in the vertebrate head, which, rather than serving for locomotion, move the eyes, control mouth operation and facilitate food uptake, speech (in humans), and facial expression (Noden 1983a; Noden and Francis-West 2006; Wachtler and Jacob 1986). Craniofacial muscles are associated with head and neck structures. In the embryo, these structures derive from the pharyngeal or branchial arches. Head muscles include the extraocular muscles, the muscles of mastication that open and close the jaw apparatus (derived from pharyngeal arch (1), and the muscles of facial expression which facilitate the movement of the lips, eyelids, and cheeks (derived from pharyngeal arch (2). The muscles of the third pharyngeal arch operate the pharynx and larynx. A number of these head muscles, including the hypobranchial muscles, the tongue muscles, and the muscles of the posterior pharyngeal arches, develop from the somites.

3 Head Muscles Are Heterogeneous in Terms of Their Mesodermal Origins

While head muscles resemble trunk muscles in term of the tissue architecture, their development is largely distinct (Bothe et al. 2007; Grifone and Kelly 2007; Kelly 2010; Sambasivan et al. 2011; Tzahor 2009; Tzahor and Evans 2011) (Fig. 1). Head muscles are highly heterogeneous in their structure, function, anatomical position, and developmental origins. In contrast to the segmented paraxial mesoderm in the trunk (the somites), the head mesoderm lacks molecular or cellular characteristics of segmentation (Noden and Trainor 2005). The head mesoderm remains mostly mesenchymal and appears homogeneous.

Muscle progenitors migrate into regions where the connective tissue progenitors may be either ectodermal (neural crest) or mesodermal in origin (Kelly 2010;

Noden and Trainor 2005; Sambasivan et al. 2011; Tzahor and Evans 2011). In the past, craniofacial development was widely viewed within the context of the neural crest cells, leading to the misconception (often seen in textbooks) that the head musculature originates from neural crest cells.

Muscles are derived from mesoderm cells (Couly et al. 1992; Harel et al. 2009; Noden 1983a). Myoblasts in the head originate within several mesodermal populations. In the head, this includes prechordal, paraxial, and splanchnic (lateral) mesoderm populations. Pharyngeal muscles are derived from the mesodermal core within the pharyngeal arches. Pharyngeal mesoderm, which contributes to the core of the pharyngeal arches, is part of the head/cranial mesoderm (Tzahor and Evans 2011) (Fig. 1). The pharyngeal mesoderm is divided into two subdomains: the loosely connected mesenchymal paraxial mesoderm, located on both sides of the neural tube and notochord and the splanchnic mesoderm, which is maintained as epithelial tissue, although there seems to be no clear division between these two mesodermal populations (Tzahor and Evans 2011). Both paraxial and splanchnic mesoderm cells converge to form the mesodermal core within the pharyngeal arches (Nathan et al. 2008). Pharyngeal mesoderm cells are found in close proximity to the pharyngeal endoderm, ectoderm, and neural crest cells, all of which influence pharyngeal muscle development (Tzahor and Evans 2011).

4 Distinct Genetic Programs in Trunk and Head Muscles

Different intrinsic and extrinsic regulatory pathways control skeletal muscle formation in the trunk and in the head (Fig. 1), as demonstrated by genetic ablation of myogenic transcription factors in mice (Harel et al. 2012; Kelly et al. 2004; Lu et al. 2002; Rudnicki et al. 1993; Tajbakhsh et al. 1997) as well as by manipulations of tissues and signaling molecules in chick embryos (Hacker and Guthrie 1998; Harel et al. 2009; Mootoosamy and Dietrich 2002; Noden et al. 1999; Tzahor et al. 2003). Skeletal muscle formation in both regions of the embryo requires either *MyoD* or *Myf5* (Rudnicki et al. 1993). It was shown later that *Myf5;MyoD* double-knockout embryos in which *Mrf4* expression is not compromised, limb and facial muscles fail to develop, whereas some trunk muscles were present (Kassar-Duchossoy et al. 2004). In contrast, mice lacking both *Pax3;Myf5* (and *Mrf4*) had no trunk muscles, yet retain normal head muscles (Tajbakhsh et al. 1997). Thus, in the absence of *Myf5*, *Pax3* is necessary for the expression of *MyoD* in the trunk, but not in the head, a finding consistent with the fact that *Pax3* is not expressed in head muscle progenitors (Hacker and Guthrie 1998; Harel et al. 2009; Tajbakhsh et al. 1997) (Fig. 1).

A recent study in mice that addressed the genetic programs promoting myogenesis in the head revealed distinct requirements for *Myf5* and *Mrf4* in extraocular muscles (EOM) and in pharyngeal muscles (Sambasivan et al. 2009). This study suggests that Tbx1 function in head muscle progenitors is similar to that of Pax3 during somitogenesis (Fig. 1). In zebrafish, the functions of Myf5 and

MyoD during head muscle formation are nonredundant: in this organism, the homeodomain transcription factor Six1 seems to play a role in the genetic program regulating development of subsets of muscles during head myogenesis (Lin et al. 2006, 2009). In contrast to this study, it was found that MyoD, but not Myf5, drives craniofacial myogenesis in zebrafish (Hinits et al. 2009). These studies suggest some interspecies differences between mouse and fish head muscle formation processes (Sambasivan et al. 2009).

The bHLH transcription factors, Tcf21 (Capsulin) and Msc (MyoR), were shown to act as upstream regulators of pharyngeal muscle development (Lu et al. 2002; Moncaut et al. 2012) (Fig. 1). In *Tcf21*/*Msc* double mutants, the masseter, pterygoid, and temporalis muscles were missing, while lower jaw muscles (e.g., anterior digastric and mylohyoid) and EOM were not affected (Lu et al. 2002). Likewise, in *Tbx1* (T-box transcription factor 1) mutants, pharyngeal muscles were frequently hypoplastic and asymmetric, whereas the EOM and tongue muscles were not affected (Grifone and Kelly 2007; Harel et al. 2012; Kelly et al. 2004). Hence, pharyngeal muscles require Tbx1 for robust bilateral specification. Head muscle defects in *Tbx1* mutants are likely due to an intrinsic defect in the pharyngeal mesoderm (Dastjerdi et al. 2007), as well as to non-cell autonomous functions of Tbx1 in the endoderm and ectoderm (Arnold et al. 2006). Analyses of Tbx1 mutant embryos indicated that several fibroblast growth factor (FGF) family members expressed in these adjacent tissues were downregulated, demonstrating a role for Tbx1 and FGF signaling during head muscle development (Hu et al. 2004; Kelly et al. 2004; Knight et al. 2008; Vitelli et al. 2002; von Scheven et al. 2006).

Tbx1 and the bicoid-related homeodomain transcription factor Pitx2 are linked to the same genetic pathway in many developmental processes, including cardiac and craniofacial muscle development (Grifone and Kelly 2007; Harel et al. 2012) (Fig. 1). In both mouse and chick, *Pitx2* is expressed in the head mesoderm and in the mesodermal core of the first pharyngeal arch (Dong et al. 2006; Shih et al. 2007) but also in other tissues. In *Pitx2* mutants, the EOM and first arch muscles are affected (Diehl et al. 2006; Shih et al. 2007). Pitx2 is expressed in trunk muscle progenitors, but it is not required for trunk myogenesis (Kitamura et al. 1999).

An important open question in the field is how the aforementioned set of transcription factors expressed in head muscle progenitors interacts in a regulatory network to activate myogenesis in the head (Fig. 1). We have recently addressed this question in the mouse by systematically analyzing single and double knockouts of several transcription factors expressed in the pharyngeal mesoderm (Harel et al. 2012). The LIM homeodomain transcription factor Lhx2 was identified as a novel player during cardiac and pharyngeal muscle development. Pharyngeal muscles were perturbed, though not completely eliminated, in knockouts of *Lhx2*, *Tbx1*, and *Tcf21*. In contrast, pharyngeal muscles were completely missing in *Tbx1;Lhx2* and *Tbx1;Myf5* double mutants. These findings indicate that a genetic circuit of Tbx1, Lhx2, and Myf5 promotes pharyngeal muscle specification. In the absence of both Myf5 and Lhx2, Tbx1 initiates myogenesis by activating *MyoD* via a parallel genetic pathway (Harel et al. 2012; Sambasivan et al. 2009). These findings suggest that a pharyngeal mesoderm regulatory network acts to ensure proper

myogenesis in the absence of single transcription factors (Fig. 1). Taken together, the Tbx1 → Lhx2 → Myf5 genetic circuit, embedded within the pharyngeal mesoderm network, regulates pharyngeal muscle specification and patterning (Harel et al. 2012).

The origins of neck muscles further demonstrate the diversity of muscle origins. These muscles, which were initially thought to be derived from the occipital somites, originate in the lateral plate mesoderm adjacent to the most anterior somites (Theis et al. 2010). These muscles display pharyngeal muscle molecular characteristics in that they are derived from cells expressing Isl1 and Tbx1 and do not require Pax3/7. As in the pharyngeal mesoderm, the expansion of myogenesis into the lateral, as opposed to paraxial, mesoderm provides another similarity to head muscles (Theis et al. 2010).

In summary, embryological and genetic studies indicate that distinct regulatory circuits control the formation of head and trunk skeletal muscles (Fig. 1). These loss-of-function studies, combined with findings from lineage tracing studies, highlight the heterogeneity in head muscle development, such that distinct genetic programs regulate different groups of muscles within the head to generate diverse muscle specifications.

5 Head Muscle Satellite Cells

Adult skeletal muscle possesses a remarkable ability to regenerate following injury. The cells that are responsible for this capacity are the satellite cells, which are adult stem cells positioned under the basal lamina of muscle fibers that can give rise to both differentiated myogenic cells, and also maintain their "stemness" by means of a self-renewal mechanism. Satellite cells play a key role in the routine maintenance, hypertrophy, and repair of adult skeletal muscles (Buckingham 2006; Kuang and Rudnicki 2008; Zammit et al. 2006).

The embryonic origins of satellite cells in the head musculature had been enigmatic. Previous studies addressing the origins of satellite cells in trunk and limb muscles (Gros et al. 2005; Kassar-Duchossoy et al. 2005; Relaix et al. 2005; Schienda et al. 2006) established that Pax3/Pax7-expressing cells within the dermomyotome compartment of somites give rise to the satellite cell pool of trunk muscles.

Lineage tracing techniques in both avian and mouse models demonstrated that pharyngeal mesoderm cells contribute to distinct pharyngeal muscles and their associated satellite cells (Harel et al. 2009). Trunk satellite cells (including tongue muscles) derive from the $Pax3^{+}$ lineage. In contrast, all head muscles and their satellite cells derive from the $MesP1^{+}$ lineage (including the tongue and EOM), whereas the Isl1 lineage marks the pharyngeal muscles and their satellite cells (Harel et al. 2009).

In addition to lineage distinction, differences in gene expression and differentiation potentials were observed between satellite cells in head compared to trunk-

derived muscles (Harel et al. 2009; Ono et al. 2010; Sambasivan et al. 2009). Transplantation of myofiber-associated head satellite cells into damaged limb muscle contributed toward efficient muscle regeneration (Harel et al. 2009; Sambasivan et al. 2009). Furthermore, in vitro experiments demonstrated the cardiogenic nature of head-, but not trunk-derived satellite cells (Harel et al. 2009). Fewer head satellite cells from the masseter are seen; also, these cells are more proliferative and display delayed differentiation relative to the timing of differentiation of satellite cells derived from trunk muscles (Ono et al. 2010). Taken together, these findings highlight a link between myogenesis in the early embryo and the generation of adult muscle progenitor pools required for muscle maintenance and regeneration.

Heterogeneity in skeletal muscles can also be seen during adulthood, as reflected in distinct genetic signatures, and susceptibilities to myopathies in both head and trunk skeletal muscles (Emery 2002; Porter et al. 2006). In humans, several diseases are characteristic of skeletal muscle tissue, and one of the longstanding mysteries in the field is why some muscles, but not others, are affected, even though they are often located in close anatomical proximity. For example, Duchenne Muscular Dystrophy (DMD), seen in 1/3,500 male births, results in lethality by the time these individuals reach their mid-twenties, even with extensive intervention and healthcare support in the later stages of the disease. Strikingly, in DMD patients, most muscles are affected; yet EOM and laryngeal muscles are largely spared. This finding reflects an underlying theme in muscle diseases: understanding why virtually all myopathies affect only a subset of muscles is of great scientific interest, with potential clinical relevance. Hence the phenotypic outcome observed in diverse myopathies maybe rooted in developmental underpinnings.

6 Head Muscle Progenitors Share Developmental History with Cardiac Progenitors

Pharyngeal mesoderm cells give rise to both pharyngeal muscles and cardiac progenitors (Grifone and Kelly 2007; Kelly 2010; Sambasivan et al. 2011; Tzahor 2009; Tzahor and Evans 2011) (Fig. 2). Studies in both chick and mouse embryos have established that pharyngeal mesoderm-derived cardiac progenitor cells, collectively referred to as the anterior heart field, populate the cardiac outflow tract, right ventricle, and atria during heart looping stages (Kelly et al. 2001; Mjaatvedt et al. 2001; Waldo et al. 2001). The anterior heart field is a subset of the second heart field. The second heart field is, therefore, the cardiogenic part of the pharyngeal mesoderm (Fig. 2). The lateral splanchnic mesoderm, known as the heart field (Fig. 2, FHF), is contiguous with the pharyngeal mesoderm, differentiates earlier, and eventually populates the left ventricle [reviewed in (Buckingham et al. 2005; Dyer and Kirby 2009; Evans et al. 2010; Tzahor and Evans 2011; Tzahor and Lassar 2001; Vincent and Buckingham 2010)]. The secondary heart field is situated

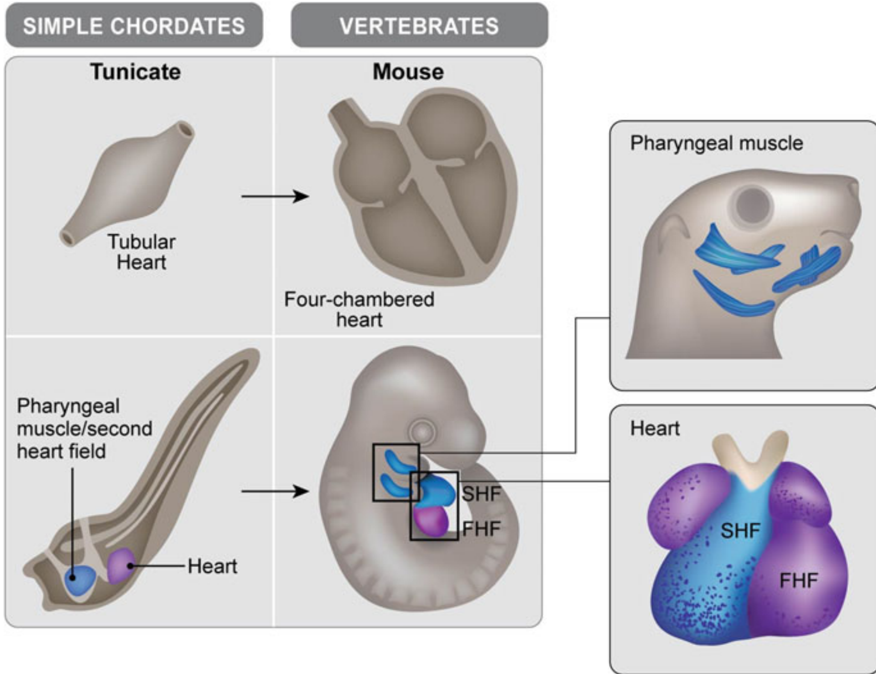


Fig. 2 The ancestral program of pharyngeal mesoderm coevolved to form the circulatory, respiratory, and feeding functions in vertebrates by modular additions of new structures and functions. Simple chordates like tunicates and other ancestral bilaterians have a tubular heart, which facilitates movement of fluids and nutrients. Hearts of reptiles, birds, and mammals have two atrial and two ventricular chambers. Striking similarities in the gene regulatory networks controlling cardiogenesis in vertebrates and tunicates are evident. Recent studies suggest that the last common ancestor of tunicates and vertebrates contained an ancestral pharyngeal mesoderm lineage that later during evolution gave rise to both heart tissue and pharyngeal muscles. The pharyngeal mesoderm ancestral program drove the coevolution of circulatory, respiratory, and feeding functions in tunicates and vertebrates. The origin of the second heart field was traced back to the anterior siphon muscle of tunicates. Second heart field (SHF) marked in *blue* and first heart field (FHF) in *purple*

slightly caudal to the anterior heart field, and gives rise to the myocardium and smooth muscle of the distal outflow tract (Kelly 2012). Hence, cells added at the arterial and venous poles of the heart derive from both overlapping and distinct regions of the pharyngeal mesoderm (Evans et al. 2010; Moorman et al. 2007).

From an embryonic point of view, the development of the head–heart region should be considered as a single morphogenetic field, in which every tissue in it is influenced by the neighboring tissues (Hutson and Kirby 2003). Due to the anatomical proximity during early embryogenesis and overlapping progenitor populations, cardiac and craniofacial birth defects are often linked (Grifone and Kelly 2007; Tzahor 2009; Tzahor and Evans 2011). DiGeorge syndrome is the most

frequent syndrome in humans (Baldini 2005; Yamagishi and Srivastava 2003). Its clinical features include cardiac defects, craniofacial, and aortic arch anomalies.

The cardiogenic potential of the pharyngeal mesoderm has been revealed over the last few years (Black 2007; Buckingham et al. 2005; Dyer and Kirby 2009; Evans et al. 2010; Tzahor and Evans 2011; Vincent and Buckingham 2010). Pharyngeal mesoderm explants dissected from early chick embryos undergo cardiogenesis in culture (Tzahor and Lassar 2001) and in vivo (Nathan et al. 2008; Rana et al. 2007; Tirosh-Finkel et al. 2006). Considerable overlap in the expression of head muscle markers [e.g., *Myf5, Tcf21 (capsulin), Msc (MyoR), Tbx1, Pitx2*] and cardiac markers such as *Islet1* and *Nkx2.5* is evident in the pharyngeal mesoderm, suggesting that these cells play a dual role in myogenesis and cardiogenesis (Bothe and Dietrich 2006; Nathan et al. 2008; Tirosh-Finkel et al. 2006). Likewise, lineage studies in the mouse demonstrated an overlap in progenitor populations contributing to pharyngeal muscles and second heart field derivatives (Dong et al. 2006; Harel et al. 2009; Nathan et al. 2008; Verzi et al. 2005) (Fig. 2).

A genetic link between Tcf21, Tbx1, and Lhx2 within the pharyngeal mesoderm was recently documented (Harel et al. 2012). Genetic ablation of these factors, alone or in combination, resulted in both cardiac and head muscle defects including DiGeorge syndrome-like phenotypes (Harel et al. 2012). Taken together, the genetic program controlling pharyngeal muscles overlaps with that of the heart.

The LIM-homeodomain protein Islet1 (Isl1) is required for a broad subset of cardiovascular progenitors in the mouse (Cai et al. 2003; Keenan et al. 2012; Laugwitz et al. 2008; Lin et al. 2007; Milgrom-Hoffman et al. 2011; Sun et al. 2007). Gene expression and lineage experiments in the chick have revealed that the core of the pharyngeal arch is divided along the proximal–distal axis, such that paraxial mesoderm cells mainly contribute to the proximal region of the core, while the splanchnic mesoderm contributes to its distal region (Nathan et al. 2008). Isl1 is expressed in the distal part of the myogenic core within the pharyngeal arches, and its expression is correlated with delayed differentiation of lower jaw muscles (Nathan et al. 2008). Overexpression of Isl1 in the chick represses pharyngeal muscle differentiation (Harel et al. 2009). Isl1 lineage-derived pharyngeal mesoderm cells were shown to contribute to a subset of pharyngeal muscles (Harel et al. 2009; Nathan et al. 2008). In conclusion, Isl1 marks a subset of pharyngeal mesoderm cells and plays an important role in the development of distinct cardiovascular and skeletal muscle progenitors (Tzahor and Evans 2011).

A retrospective clonal analysis in the mouse demonstrated that head muscles and cardiomyocytes, derived from the second heart field, originate from bipotent pharyngeal mesoderm progenitors (Lescroart et al. 2010). In conclusion, recent studies provide cellular and molecular insights into how pharyngeal mesoderm cells form distinct pharyngeal arch-derived muscles and certain parts of the heart (Fig. 2).

7 Evolution of Pharyngeal Mesoderm: From Pharyngeal Arch-Derived Muscles to the Heart

The architecture and function of muscle cells have been remarkably conserved throughout evolution, suggesting that all muscle cells likely evolved from an ancestral developmental program involving a single contractile myogenic cell type (Baugh and Hunter 2006; Fukushige et al. 2006). The appearance of the head and neck during evolution is part of the adaption of vertebrates from filter feeding to active predation and other forms of food intake. Numerous head muscle specializations have arisen to accommodate or permit a wide range of craniofacial adaptations and functions, for example, to changes in nutrition availabilities. The current notion holds that craniofacial muscles are considered to be a novel structure that appeared relatively late in evolution with the emergence of gnathostomes (vertebrates with jaws) and that head muscles evolved independently of trunk skeletal muscles (Sambasivan et al. 2011).

Cellular and molecular parallels between cardiac and pharyngeal muscles are probably more ancient in evolution and could go back 600–700 million years (Grifone and Kelly 2007; Olson 2006; Sambasivan et al. 2011; Tzahor 2009; Tzahor and Evans 2011). The fact that the developmental programs of the heart and pharyngeal muscles are tightly linked suggests that these tissues share common evolutionary origins. For example, nematodes are invertebrates that do not possess a heart or defined circulatory system. Instead, their pharyngeal muscles function like a heart, exhibit electrical activity similar to mammalian cardiomyocytes, and are regulated by the homeobox gene *Nkx2.5* (ceh-22) (Harfe and Fire 1998), reviewed in (Grifone and Kelly 2007; Olson 2006; Sambasivan et al. 2011; Tzahor 2009; Tzahor and Evans 2011).

Recent studies in tunicates provide a broader understanding of the cardio-craniofacial muscle evolution. Tunicates belong to the *Chordata* phylum, and are considered as the "sister group" of vertebrates. The tunicate *Ciona intestinalis* is a sessile marine invertebrate (Fig. 2). As in vertebrates, the *Ciona* heart is located ventrally and posterior to the pharynx and anterior to the stomach; in the gastrulating embryo, its heart arises from a pair of blastomeres expressing the MesP gene. Several studies suggest significant similarities in the gene regulatory networks controlling cardiogenesis in vertebrates and tunicates (Davidson 2007; Satou et al. 2004; Tolkin and Christiaen 2012). The heart and pharyngeal muscle cells in Ciona are derived from $MesP^{+}$ cells. Strikingly, $Isl1^{+}$ cells in Ciona (Stolfi et al. 2010) as in vertebrates (Harel et al. 2009; Nathan et al. 2008) give rise to pharyngeal muscles (termed siphon muscles in *Ciona*). These findings suggest that the last common ancestor of tunicates and vertebrates contained pharyngeal mesoderm ancestral progenitors derived from $MesP^{+}$ lineages that expressed Isl1, FoxF, and Nkx2.5 and had the potential to give rise to both heart tissue and pharyngeal muscles (Fig. 2).

How did these cells acquire their cell fate identity (heart or siphon muscle)? A recent study in *Ciona intestinalis* revealed that Nkx2.5 promotes early heart

specification by inhibiting the formation of pharyngeal muscles. Conversely, Tbx1 induces pharyngeal muscle fate by activating COE (Collier/Olf-1/EBF) on one hand and inhibiting Gata-induced cardiogenesis on the other hand (Wang et al. 2013). Hence, a cross-repression mechanism of transcription factors within the pharyngeal mesoderm underlies a cell fate switch between heart and pharyngeal muscle in a conserved lineage of cardio-craniofacial (termed cardiopharyngeal in tunicates) muscle progenitors. Extrinsic signaling mechanisms could also promote this cell fate segregation. BMP signaling was shown to promote cardiogenesis at the expense of skeletal muscle differentiation (Tirosh-Finkel et al. 2006; Tzahor et al. 2003).

With the increasing complexity of the vertebrate heart and, in particular, during the heart tube elongation that occurs in vertebrates, Isl1^{+} pharyngeal mesoderm cells were recruited into the looping heart to give rise to cardiomyocytes. This study suggests that reallocation of the pharyngeal mesoderm module into the looping heart represents the emergence of the second heart field in vertebrates (Fig. 2). In addition, these findings suggest a distinct evolutionary separation in the origins of the two heart fields.

8 Extrinsic Regulation of Head Muscle Development

The tissues and signaling molecules that promote skeletal muscle formation in the somites have been intensively studied (Buckingham 2006; Pourquie 2001; Tajbakhsh 2005). Myogenesis in the trunk is affected by signals emanating from the axial tissues, the surface ectoderm, and the lateral plate mesoderm. Wnt family members expressed in the dorsal neural tube work together with Sonic hedgehog (Shh) expressed in the notochord to activate *Myf*5 and *MyoD* in the somite (Borycki et al. 2000; Gustafsson et al. 2002; Munsterberg et al. 1995; Stern et al. 1995; Tajbakhsh et al. 1998). Bone morphogenetic protein (BMP) signals from the lateral plate have been shown to delay the activation of myogenic bHLH gene expression in the somites (Pourquie et al. 1996; Reshef et al. 1998).

Although signals from the dorsal neural tube promote myogenesis in the trunk (Munsterberg et al. 1995), such signals block myogenesis in pharyngeal mesoderm explants (Tzahor et al. 2003). Accordingly, overexpression of Wnt family members expressed in either the dorsal neural tube (*Wnt3a*) or surface ectoderm (*Wnt13*), or forced expression of stabilized β-*catenin*, which stimulates the canonical Wnt signaling pathway, repress myogenesis in the chick (Tzahor et al. 2003). In contrast, Wnt family members expressed in either the dorsal neural tube or in surface ectoderm overlying the somites were shown to promote skeletal myogenesis in this tissue (Capdevila et al. 1998; Ikeya and Takada 1998; Munsterberg et al. 1995; Stern et al. 1995; Tajbakhsh et al. 1998; Takada et al. 1994).

BMP signals were found to repress myogenesis in both head (Tirosh-Finkel et al. 2006; Tzahor et al. 2003) and trunk regions (Amthor et al. 1999; Hirsinger et al. 1997; McMahon et al. 1998; Pourquie et al. 1996; Reshef et al. 1998).

Accordingly, myogenesis in the head is induced by a combination of BMP inhibitors such as Noggin and Gremlin and a Wnt inhibitor (e.g., Frzb). These molecules were shown to be secreted by both cranial neural crest cells and by other tissues surrounding the myogenic progenitors (Tzahor et al. 2003).

FGF signaling affects skeletal muscle progenitors in several ways, promoting both progenitor cell proliferation and their differentiation depending on the cellular and spatiotemporal context. Myoblasts grown in culture start to differentiate, when the amount of growth factors in the media is reduced. The key growth factor repressing myogenic differentiation in these cultures was found to be FGF (Clegg et al. 1987; Olwin and Rapraeger 1992). In the chick embryo, Fgfr4 is expressed in Myf5+ MyoD + myogenic cells in the limb (Marcelle et al. 1995); in the mouse, this gene is directly regulated by Pax3 (Lagha et al. 2008). Forced expression of Fgf8 in chick somites upregulated Fgfr4 expression and enhanced myogenic differentiation. Likewise, electroporation of a dominant-negative Fgfr4 inhibited myogenic differentiation (Marics et al. 2002). Together, these in vivo studies suggest that FGF signaling is required for trunk myogenesis. In the head region, the expression of certain FGF family members is dependent on Tbx1 function, although the precise role of FGF signaling on head myogenesis is not entirely clear (Knight et al. 2008; von Scheven et al. 2006).

Taken together, in the trunk, signals from the neural tube and notochord specifically stimulate the development of the epaxial muscle anlagen, which remain in the vicinity of the axial midline tissues to give rise to the deep muscles of the back (Burke and Nowicki 2003). In contrast, head muscles develop at a distance from the neural tube (the developing brain) in either the core of the pharyngeal arches or around the eye (extraocular muscles). Wnt, BMP and FGF signals block premature head muscle differentiation in the vicinity of the axial tissues. It is tempting to speculate that these signals play a role in the delayed differentiation of head muscle progenitors within regulatory circuits involving transcription factors such as *Tbx1, Pitx2, MyoR, Capsulin*, and *Isl1*. Overexpression studies in chick pharyngeal mesoderm explants and in vivo have demonstrated that this is, indeed, the case (Harel et al. 2009). Thus, Wnt BMP and FGF signaling pathways are thought to control the balance between myogenic precursor proliferation and differentiation in the head. Whether some of these extrinsic signals play roles in the specification of head muscle progenitors is a plausible assumption that remains to be validated.

9 Cranial Neural Crest Cells Affect Head Muscle Patterning and Differentiation

Cranial neural crest cells surround the muscle *anlagen* in a highly organized fashion, separating the myoblasts from the overlying surface ectoderm (Noden 1983b; Trainor et al. 1994). Cranial neural crest cells give rise to most of the skeletal elements of the head and also serve as precursors for connective tissues and

tendons (Couly et al. 1993; Le Douarin et al. 1993). Mesoderm-derived muscle progenitors fuse together to form a myofiber, which is attached to a specific cranial neural crest-derived skeletal element through cranial neural crest-derived connective tissue in a precisely coordinated manner. Neural crest cells affect the patterning of muscle, placodes, and connective tissue in the head. In a similar fashion, cranial neural crest regulates EOM formation within the orbit (Bohnsack et al. 2011).

Craniofacial shapes are amazingly diverse in vertebrates (Helms et al. 2005). This diversity apparently reflects a tight linkage between the skeletal elements (cranial neural crest), connective tissue (crest), and muscles (mesoderm). The relationship between muscle and skeletal elements within the jaw region controls feeding mechanics. This may reflect on the ability of vertebrates to rapidly modify the jaw complex, a critical evolutionary advantage enabling the organism to accommodate to new ecological conditions (Herrel et al. 2005). In keeping with this view, the emergence of vertebrate predators is also associated with the increased muscularization of pharyngeal muscles, along with an increase in size of the jaw skeleton (Takio et al. 2004).

The degree to which skeletal muscle specification, differentiation, and patterning is intrinsic to muscle (mesoderm) progenitors, or controlled by extrinsic environmental signals (e.g., cranial neural crest cells), is a fundamental embryological question. It has long been suggested that, in addition to contributing to the formation of skeletal elements and connective tissue in the head, cranial neural crest cells may also be involved in the patterning of the head musculature (Couly et al. 1992; Ericsson et al. 2004; Grammatopoulos et al. 2000; Grenier et al. 2009; Heude et al. 2010; Kontges and Lumsden 1996; Noden 1983a, b; Olsson et al. 2001; Rinon et al. 2007; Schilling and Kimmel 1997; Tokita and Schneider 2009; Tzahor et al. 2003).

Because skeletal muscles in the head, except EOM, still form (albeit in a distorted fashion), following in vivo ablation of the cranial neural crest cells in amphibian and chick embryos [(Ericsson et al. 2004; Olsson et al. 2001; Tzahor et al. 2003; von Scheven et al. 2006)], the precise impact of cranial neural crest cells on head muscle formation remains unclear. Thus, while it is generally accepted that that cranial neural crest cells influence head muscle formation, exactly how has yet to be elucidated. The current view in the field is that cranial neural crest-derived connective tissue progressively imposes the characteristic anatomical musculoskeletal architecture upon muscle progenitors (Heude et al. 2010; Rinon et al. 2007; Tokita and Schneider 2009).

Pharyngeal mesoderm progenitors are exposed to signals from pharyngeal arch endoderm, ectoderm, and neural crest cells that together create a complex regulatory system [reviewed in (Rochais et al. 2009; Vincent and Buckingham 2010)]. Perturbation of the balance of signals within this system can lead to abnormal cardiac and craniofacial development. Neural crest ablation in the chick, for example, results in increased FGF signaling and elevated proliferation in the pharyngeal mesoderm (Hutson et al. 2006; Rinon et al. 2007; Waldo et al. 2005). These findings suggest that both cardiac neural crest (affecting caudal pharyngeal mesoderm progenitors) and cranial neural crest cells (affecting cranial pharyngeal

mesoderm) buffer proliferative signals secreted from the endoderm and ectoderm to promote PM migration and differentiation.

Acknowledgements The research in Prof. Tzahor's lab is supported by grants from the European Research Council and Israel Science Foundation.

References

Amthor H, Christ B, Patel K (1999) A molecular mechanism enabling continuous embryonic muscle growth—a balance between proliferation and differentiation. Development 126:1041–1053

Arnold JS, Werling U, Braunstein EM, Liao J, Nowotschin S, Edelmann W, Hebert JM, Morrow BE (2006) Inactivation of Tbx1 in the pharyngeal endoderm results in 22q11DS malformations. Development 133:977–987

Baldini A (2005) Dissecting contiguous gene defects: TBX1. Curr Opin Genet Dev 15:279–284

Baugh LR, Hunter CP (2006) MyoD, modularity, and myogenesis: conservation of regulators and redundancy in C. elegans. Genes Dev 20:3342–3346

Berkes CA, Tapscott SJ (2005) MyoD and the transcriptional control of myogenesis. Semin Cell Dev Biol 16:585–595

Black BL (2007) Transcriptional pathways in second heart field development. Semin Cell Dev Biol 18:67–76

Bohnsack BL, Gallina D, Thompson H, Kasprick DS, Lucarelli MJ, Dootz G, Nelson C, McGonnell IM, Kahana A (2011) Development of extraocular muscles require early signals from periocular neural crest and the developing eye. Arch Ophthalmol 129:1030–1041

Borycki A, Brown AM, Emerson CP Jr (2000) Shh and Wnt signaling pathways converge to control Gli gene activation in avian somites. Development 127:2075–2087

Bothe I, Dietrich S (2006) The molecular setup of the avian head mesoderm and its implication for craniofacial myogenesis. Dev Dyn 235:2845–2860

Bothe I, Ahmed MU, Winterbottom FL, von Scheven G, Dietrich S (2007) Extrinsic versus intrinsic cues in avian paraxial mesoderm patterning and differentiation. Dev Dyn 236:2397–2409

Bryson-Richardson RJ, Currie PD (2008) The genetics of vertebrate myogenesis. Nat Rev Genet 9:632–646

Buckingham M (2001) Skeletal muscle formation in vertebrates. Curr Opin Genet Dev 11:440–448

Buckingham M (2006) Myogenic progenitor cells and skeletal myogenesis in vertebrates. Curr Opin Genet Dev 16:525–532

Buckingham M, Vincent SD (2009) Distinct and dynamic myogenic populations in the vertebrate embryo. Curr Opin Genet Dev 19:444–453

Buckingham M, Meilhac S, Zaffran S (2005) Building the mammalian heart from two sources of myocardial cells. Nat Rev Genet 6:826–835

Burke AC, Nowicki JL (2003) A new view of patterning domains in the vertebrate mesoderm. Dev Cell 4:159–165

Cai CL, Liang X, Shi Y, Chu PH, Pfaff SL, Chen J, Evans S (2003) Isl1 identifies a cardiac progenitor population that proliferates prior to differentiation and contributes a majority of cells to the heart. Dev Cell 5:877–889

Capdevila J, Tabin C, Johnson RL (1998) Control of dorsoventral somite patterning by Wnt-1 and beta-catenin. Dev Biol 193:182–194

Christ B, Ordahl CP (1995) Early stages of chick somite development. Anat Embryol (Berl) 191:381–396

Clegg CH, Linkhart TA, Olwin BB, Hauschka SD (1987) Growth factor control of skeletal muscle differentiation: commitment to terminal differentiation occurs in G1 phase and is repressed by fibroblast growth factor. J Cell Biol 105:949–956
Couly GF, Coltey PM, Le Douarin NM (1992) The developmental fate of the cephalic mesoderm in quail-chick chimeras. Development 114:1–15
Couly GF, Coltey PM, Le Douarin NM (1993) The triple origin of skull in higher vertebrates: a study in quail-chick chimeras. Development 117:409–429
Dastjerdi A, Robson L, Walker R, Hadley J, Zhang Z, Rodriguez-Niedenfuhr M, Ataliotis P, Baldini A, Scambler P, Francis-West P (2007) Tbx1 regulation of myogenic differentiation in the limb and cranial mesoderm. Dev Dyn 236:353–363
Davidson B (2007) Ciona intestinalis as a model for cardiac development. Semin Cell Dev Biol 18:16–26
Diehl AG, Zareparsi S, Qian M, Khanna R, Angeles R, Gage PJ (2006) Extraocular muscle morphogenesis and gene expression are regulated by Pitx2 gene dose. Invest Ophthalmol Vis Sci 47:1785–1793
Dong F, Sun X, Liu W, Ai D, Klysik E, Lu MF, Hadley J, Antoni L, Chen L, Baldini A, Francis-West P, Martin JF (2006) Pitx2 promotes development of splanchnic mesoderm-derived branchiomeric muscle. Development 133:4891–4899
Dyer LA, Kirby ML (2009) The role of secondary heart field in cardiac development. Dev Biol 336:137–144
Emery AE (2002) The muscular dystrophies. Lancet 359:687–695
Ericsson R, Cerny R, Falck P, Olsson L (2004) Role of cranial neural crest cells in visceral arch muscle positioning and morphogenesis in the Mexican axolotl, Ambystoma mexicanum. Dev Dyn 231:237–247
Evans SM, Yelon D, Conlon FL, Kirby ML (2010) Myocardial lineage development. Circ Res 107:1428–1444
Fukushige T, Brodigan TM, Schriefer LA, Waterston RH, Krause M (2006) Defining the transcriptional redundancy of early bodywall muscle development in C. elegans: evidence for a unified theory of animal muscle development. Genes Dev 20:3395–3406
Grammatopoulos GA, Bell E, Toole L, Lumsden A, Tucker AS (2000) Homeotic transformation of branchial arch identity after Hoxa2 overexpression. Development 127:5355–5365
Grenier J, Teillet MA, Grifone R, Kelly RG, Duprez D (2009) Relationship between neural crest cells and cranial mesoderm during head muscle development. PLoS One 4:e4381
Grifone R, Kelly RG (2007) Heartening news for head muscle development. Trends Genet 23:365–369
Gros J, Manceau M, Thome V, Marcelle C (2005) A common somitic origin for embryonic muscle progenitors and satellite cells. Nature 435:954–958
Gustafsson MK, Pan H, Pinney DF, Liu Y, Lewandowski A, Epstein DJ, Emerson CP Jr (2002) Myf5 is a direct target of long-range Shh signaling and Gli regulation for muscle specification. Genes Dev 16:114–126
Hacker A, Guthrie S (1998) A distinct developmental programme for the cranial paraxial mesoderm in the chick embryo. Development 125:3461–3472
Harel I, Nathan E, Tirosh-Finkel L, Zigdon H, Guimaraes-Camboa N, Evans SM, Tzahor E (2009) Distinct origins and genetic programs of head muscle satellite cells. Dev Cell 16:822–832
Harel I, Maezawa Y, Avraham R, Rinon A, Ma HY, Cross JW, Leviatan N, Hegesh J, Roy A, Jacob-Hirsch J, Rechavi G, Carvajal J, Tole S, Kioussi C, Quaggin S, Tzahor E (2012) Pharyngeal mesoderm regulatory network controls cardiac and head muscle morphogenesis. Proc Natl Acad Sci U S A 109:18839–18844
Harfe BD, Fire A (1998) Muscle and nerve-specific regulation of a novel NK-2 class homeodomain factor in Caenorhabditis elegans. Development 125:421–429
Helms JA, Cordero D, Tapadia MD (2005) New insights into craniofacial morphogenesis. Development 132:851–861

Herrel A, Podos J, Huber SK, Hendry AP (2005) Evolution of bite force in Darwin's finches: a key role for head width. J Evol Biol 18:669–675

Heude E, Bouhali K, Kurihara Y, Kurihara H, Couly G, Janvier P, Levi G (2010) Jaw muscularization requires Dlx expression by cranial neural crest cells. Proc Natl Acad Sci U S A 107:11441–11446

Hinits Y, Osborn DP, Hughes SM (2009) Differential requirements for myogenic regulatory factors distinguish medial and lateral somitic, cranial and fin muscle fibre populations. Development 136:403–414

Hirsinger E, Duprez D, Jouve C, Malapert P, Cooke J, Pourquie O (1997) Noggin acts downstream of Wnt and Sonic Hedgehog to antagonize BMP4 in avian somite patterning. Development 124:4605–4614

Hu T, Yamagishi H, Maeda J, McAnally J, Yamagishi C, Srivastava D (2004) Tbx1 regulates fibroblast growth factors in the anterior heart field through a reinforcing autoregulatory loop involving forkhead transcription factors. Development 131:5491–5502

Hutson MR, Kirby ML (2003) Neural crest and cardiovascular development: a 20-year perspective. Birth Defects Res C Embryo Today 69:2–13

Hutson MR, Zhang P, Stadt HA, Sato AK, Li YX, Burch J, Creazzo TL, Kirby ML (2006) Cardiac arterial pole alignment is sensitive to FGF8 signaling in the pharynx. Dev Biol 295:486–497

Ikeya M, Takada S (1998) Wnt signaling from the dorsal neural tube is required for the formation of the medial dermomyotome. Development 125:4969–4976

Kassar-Duchossoy L, Gayraud-Morel B, Gomes D, Rocancourt D, Buckingham M, Shinin V, Tajbakhsh S (2004) Mrf4 determines skeletal muscle identity in Myf5:Myod double-mutant mice. Nature 431:466–471

Kassar-Duchossoy L, Giacone E, Gayraud-Morel B, Jory A, Gomes D, Tajbakhsh S (2005) Pax3/Pax7 mark a novel population of primitive myogenic cells during development. Genes Dev 19:1426–1431

Keenan ID, Rhee HJ, Chaudhry B, Henderson DJ (2012) Origin of non-cardiac endothelial cells from an Isl1+ lineage. FEBS Lett 586:1790–1794

Kelly RG (2010) Core issues in craniofacial myogenesis. Exp Cell Res 316:3034–3041

Kelly RG (2012) The second heart field. Curr Top Dev Biol 100:33–65

Kelly RG, Brown NA, Buckingham ME (2001) The arterial pole of the mouse heart forms from Fgf10-expressing cells in pharyngeal mesoderm. Dev Cell 1:435–440

Kelly RG, Jerome-Majewska LA, Papaioannou VE (2004) The del22q11.2 candidate gene Tbx1 regulates branchiomeric myogenesis. Hum Mol Genet 13:2829–2840

Kitamura K, Miura H, Miyagawa-Tomita S, Yanazawa M, Katoh-Fukui Y, Suzuki R, Ohuchi H, Suehiro A, Motegi Y, Nakahara Y, Kondo S, Yokoyama M (1999) Mouse Pitx2 deficiency leads to anomalies of the ventral body wall, heart, extra- and periocular mesoderm and right pulmonary isomerism. Development 126:5749–5758

Knight RD, Mebus K, Roehl HH (2008) Mandibular arch muscle identity is regulated by a conserved molecular process during vertebrate development. J Exp Zoolog B Mol Dev Evol 310:355–369

Kontges G, Lumsden A (1996) Rhombencephalic neural crest segmentation is preserved throughout craniofacial ontogeny. Development 122:3229–3242

Kuang S, Rudnicki MA (2008) The emerging biology of satellite cells and their therapeutic potential. Trends Mol Med 14:82–91

Lagha M, Kormish JD, Rocancourt D, Manceau M, Epstein JA, Zaret KS, Relaix F, Buckingham ME (2008) Pax3 regulation of FGF signaling affects the progression of embryonic progenitor cells into the myogenic program. Genes Dev 22:1828–1837

Laugwitz KL, Moretti A, Caron L, Nakano A, Chien KR (2008) Islet1 cardiovascular progenitors: a single source for heart lineages? Development 135:193–205

Le Douarin NM, Ziller C, Couly GF (1993) Patterning of neural crest derivatives in the avian embryo: in vivo and in vitro studies. Dev Biol 159:24–49

Lescroart F, Kelly RG, Le Garrec JF, Nicolas JF, Meilhac SM, Buckingham M (2010) Clonal analysis reveals common lineage relationships between head muscles and second heart field derivatives in the mouse embryo. Development 137:3269–3279

Lin CY, Yung RF, Lee HC, Chen WT, Chen YH, Tsai HJ (2006) Myogenic regulatory factors Myf5 and Myod function distinctly during craniofacial myogenesis of zebrafish. Dev Biol 299:594–608

Lin L, Cui L, Zhou W, Dufort D, Zhang X, Cai CL, Bu L, Yang L, Martin J, Kemler R, Rosenfeld MG, Chen J, Evans SM (2007) beta-Catenin directly regulates Islet1 expression in cardiovascular progenitors and is required for multiple aspects of cardiogenesis. Proc Natl Acad Sci U S A 104:9313–9318

Lin CY, Chen WT, Lee HC, Yang PH, Yang HJ, Tsai HJ (2009) The transcription factor Six1a plays an essential role in the craniofacial myogenesis of zebrafish. Dev Biol 331:152–166

Lu JR, Bassel-Duby R, Hawkins A, Chang P, Valdez R, Wu H, Gan L, Shelton JM, Richardson JA, Olson EN (2002) Control of facial muscle development by MyoR and capsulin. Science 298:2378–2381

Marcelle C, Wolf J, Bronner-Fraser M (1995) The in vivo expression of the FGF receptor FREK mRNA in avian myoblasts suggests a role in muscle growth and differentiation. Dev Biol 172:100–114

Marics I, Padilla F, Guillemot JF, Scaal M, Marcelle C (2002) FGFR4 signaling is a necessary step in limb muscle differentiation. Development 129:4559–4569

McMahon JA, Takada S, Zimmerman LB, Fan CM, Harland RM, McMahon AP (1998) Noggin-mediated antagonism of BMP signaling is required for growth and patterning of the neural tube and somite. Genes Dev 12:1438–1452

Milgrom-Hoffman M, Harrelson Z, Ferrara N, Zelzer E, Evans SM, Tzahor E (2011) The heart endocardium is derived from vascular endothelial progenitors. Development 138:4777–4787

Mjaatvedt CH, Nakaoka T, Moreno-Rodriguez R, Norris RA, Kern MJ, Eisenberg CA, Turner D, Markwald RR (2001) The outflow tract of the heart is recruited from a novel heart-forming field. Dev Biol 238:97–109

Moncaut N, Cross JW, Siligan C, Keith A, Taylor K, Rigby PW, Carvajal JJ (2012) Musculin and TCF21 coordinate the maintenance of myogenic regulatory factor expression levels during mouse craniofacial development. Development 139:958–967

Moorman AF, Christoffels VM, Anderson RH, van den Hoff MJ (2007) The heart-forming fields: one or multiple? Philos Trans R Soc Lond B Biol Sci 362:1257–1265

Mootoosamy RC, Dietrich S (2002) Distinct regulatory cascades for head and trunk myogenesis. Development 129:573–583

Munsterberg AE, Kitajewski J, Bumcrot DA, McMahon AP, Lassar AB (1995) Combinatorial signaling by Sonic hedgehog and Wnt family members induces myogenic bHLH gene expression in the somite. Genes Dev 9:2911–2922

Nathan E, Monovich A, Tirosh-Finkel L, Harrelson Z, Rousso T, Rinon A, Harel I, Evans SM, Tzahor E (2008) The contribution of Islet1-expressing splanchnic mesoderm cells to distinct branchiomeric muscles reveals significant heterogeneity in head muscle development. Development 135:647–657

Noden DM (1983a) The embryonic origins of avian cephalic and cervical muscles and associated connective tissues. Am J Anat 168:257–276

Noden DM (1983b) The role of the neural crest in patterning of avian cranial skeletal, connective, and muscle tissues. Dev Biol 96:144–165

Noden DM, Francis-West P (2006) The differentiation and morphogenesis of craniofacial muscles. Dev Dyn 235:1194–1218

Noden DM, Trainor PA (2005) Relations and interactions between cranial mesoderm and neural crest populations. J Anat 207:575–601

Noden DM, Marcucio R, Borycki AG, Emerson CP Jr (1999) Differentiation of avian craniofacial muscles: I. Patterns of early regulatory gene expression and myosin heavy chain synthesis. Dev Dyn 216:96–112

Olson EN (2006) Gene regulatory networks in the evolution and development of the heart. Science 313:1922–1927

Olsson L, Falck P, Lopez K, Cobb J, Hanken J (2001) Cranial neural crest cells contribute to connective tissue in cranial muscles in the anuran amphibian, Bombina orientalis. Dev Biol 237:354–367

Olwin BB, Rapraeger A (1992) Repression of myogenic differentiation by aFGF, bFGF, and K-FGF is dependent on cellular heparan sulfate. J Cell Biol 118:631–639

Ono Y, Boldrin L, Knopp P, Morgan JE, Zammit PS (2010) Muscle satellite cells are a functionally heterogeneous population in both somite-derived and branchiomeric muscles. Dev Biol 337:29–41

Porter JD, Israel S, Gong B, Merriam AP, Feuerman J, Khanna S, Kaminski HJ (2006) Distinctive morphological and gene/protein expression signatures during myogenesis in novel cell lines from extraocular and hindlimb muscle. Physiol Genomics 24:264–275

Pourquie O (2001) Vertebrate somitogenesis. Annu Rev Cell Dev Biol 17:311–350

Pourquie O, Fan CM, Coltey M, Hirsinger E, Watanabe Y, Breant C, Francis-West P, Brickell P, Tessier-Lavigne M, Le Douarin NM (1996) Lateral and axial signals involved in avian somite patterning: a role for BMP4. Cell 84:461–471

Rana MS, Horsten NC, Tesink-Taekema S, Lamers WH, Moorman AF, van den Hoff MJ (2007) Trabeculated right ventricular free wall in the chicken heart forms by ventricularization of the myocardium initially forming the outflow tract. Circ Res 100:1000–1007

Relaix F, Rocancourt D, Mansouri A, Buckingham M (2005) A Pax3/Pax7-dependent population of skeletal muscle progenitor cells. Nature 435:948–953

Reshef R, Maroto M, Lassar AB (1998) Regulation of dorsal somitic cell fates: BMPs and Noggin control the timing and pattern of myogenic regulator expression. Genes Dev 12:290–303

Rinon A, Lazar S, Marshall H, Buchmann-Moller S, Neufeld A, Elhanany-Tamir H, Taketo MM, Sommer L, Krumlauf R, Tzahor E (2007) Cranial neural crest cells regulate head muscle patterning and differentiation during vertebrate embryogenesis. Development 134:3065–3075

Rochais F, Mesbah K, Kelly RG (2009) Signaling pathways controlling second heart field development. Circ Res 104:933–942

Rudnicki MA, Schnegelsberg PN, Stead RH, Braun T, Arnold HH, Jaenisch R (1993) MyoD or Myf-5 is required for the formation of skeletal muscle. Cell 75:1351–1359

Sambasivan R, Gayraud-Morel B, Dumas G, Cimper C, Paisant S, Kelly R, Tajbakhsh S (2009) Distinct regulatory cascades govern extraocular and pharyngeal arch muscle progenitor cell fates. Dev Cell 16:810–821

Sambasivan R, Kuratani S, Tajbakhsh S (2011) An eye on the head: the development and evolution of craniofacial muscles. Development 138:2401–2415

Satou Y, Imai KS, Satoh N (2004) The ascidian Mesp gene specifies heart precursor cells. Development 131:2533–2541

Schienda J, Engleka KA, Jun S, Hansen MS, Epstein JA, Tabin CJ, Kunkel LM, Kardon G (2006) Somitic origin of limb muscle satellite and side population cells. Proc Natl Acad Sci U S A 103:945–950

Schilling TF, Kimmel CB (1997) Musculoskeletal patterning in the pharyngeal segments of the zebrafish embryo. Development 124:2945–2960

Shih HP, Gross MK, Kioussi C (2007) Cranial muscle defects of Pitx2 mutants result from specification defects in the first branchial arch. Proc Natl Acad Sci U S A 104:5907–5912

Stern HM, Brown AM, Hauschka SD (1995) Myogenesis in paraxial mesoderm: preferential induction by dorsal neural tube and by cells expressing Wnt-1. Development 121:3675–3686

Stolfi A, Gainous TB, Young JJ, Mori A, Levine M, Christiaen L (2010) Early chordate origins of the vertebrate second heart field. Science 329:565–568

Sun Y, Liang X, Najafi N, Cass M, Lin L, Cai CL, Chen J, Evans SM (2007) Islet 1 is expressed in distinct cardiovascular lineages, including pacemaker and coronary vascular cells. Dev Biol 304:286–296

Tajbakhsh S (2005) Skeletal muscle stem and progenitor cells: reconciling genetics and lineage. Exp Cell Res 306:364–372

Tajbakhsh S, Rocancourt D, Cossu G, Buckingham M (1997) Redefining the genetic hierarchies controlling skeletal myogenesis: Pax-3 and Myf-5 act upstream of MyoD. Cell 89:127–138

Tajbakhsh S, Borello U, Vivarelli E, Kelly R, Papkoff J, Duprez D, Buckingham M, Cossu G (1998) Differential activation of Myf5 and MyoD by different Wnts in explants of mouse paraxial mesoderm and the later activation of myogenesis in the absence of Myf5. Development 125:4155–4162

Takada S, Stark KL, Shea MJ, Vassileva G, McMahon JA, McMahon AP (1994) Wnt-3A regulates somite and tailbud formation in the mouse embryo. Genes and Development 8:174–189

Takio Y, Pasqualetti M, Kuraku S, Hirano S, Rijli FM, Kuratani S (2004) Evolutionary biology: lamprey Hox genes and the evolution of jaws. Nature 429:1 p following 262

Theis S, Patel K, Valasek P, Otto A, Pu Q, Harel I, Tzahor E, Tajbakhsh S, Christ B, Huang R (2010) The occipital lateral plate mesoderm is a novel source for vertebrate neck musculature. Development 137:2961–2971

Tirosh-Finkel L, Elhanany H, Rinon A, Tzahor E (2006) Mesoderm progenitor cells of common origin contribute to the head musculature and the cardiac outflow tract. Development 133:1943–1953

Tokita M, Schneider RA (2009) Developmental origins of species-specific muscle pattern. Dev Biol 331:311–325

Tolkin T, Christiaen L (2012) Development and evolution of the ascidian cardiogenic mesoderm. Curr Top Dev Biol 100:107–142

Trainor PA, Tan SS, Tam PP (1994) Cranial paraxial mesoderm: regionalisation of cell fate and impact on craniofacial development in mouse embryos. Development 120:2397–2408

Tzahor E (2009) Heart and craniofacial muscle development: a new developmental theme of distinct myogenic fields. Dev Biol 327:273–279

Tzahor E, Evans SM (2011) Pharyngeal mesoderm development during embryogenesis: implications for both heart and head myogenesis. Cardiovasc Res. 91:196–202

Tzahor E, Lassar AB (2001) Wnt signals from the neural tube block ectopic cardiogenesis. Genes Dev 15:255–260

Tzahor E, Kempf H, Mootoosamy RC, Poon AC, Abzhanov A, Tabin CJ, Dietrich S, Lassar AB (2003) Antagonists of Wnt and BMP signaling promote the formation of vertebrate head muscle. Genes Dev 17:3087–3099

Verzi MP, McCulley DJ, De Val S, Dodou E, Black BL (2005) The right ventricle, outflow tract, and ventricular septum comprise a restricted expression domain within the secondary/anterior heart field. Dev Biol 287:134–145

Vincent SD, Buckingham ME (2010) How to make a heart: the origin and regulation of cardiac progenitor cells. Curr Top Dev Biol 90:1–41

Vitelli F, Taddei I, Morishima M, Meyers EN, Lindsay EA, Baldini A (2002) A genetic link between Tbx1 and fibroblast growth factor signaling. Development 129:4605–4611

von Scheven G, Alvares LE, Mootoosamy RC, Dietrich S (2006) Neural tube derived signals and Fgf8 act antagonistically to specify eye versus mandibular arch muscles. Development 133:2731–2745

Wachtler F, Jacob M (1986) Origin and development of the cranial skeletal muscles. Bibl Anat (29); 24–46

Waldo KL, Kumiski DH, Wallis KT, Stadt HA, Hutson MR, Platt DH, Kirby ML (2001) Conotruncal myocardium arises from a secondary heart field. Development 128:3179–3188

Waldo KL, Hutson MR, Stadt HA, Zdanowicz M, Zdanowicz J, Kirby ML (2005) Cardiac neural crest is necessary for normal addition of the myocardium to the arterial pole from the secondary heart field. Dev Biol 281:66–77

Wang W, Razy-Krajka F, Siu E, Ketcham A, Christiaen L (2013) NK4 antagonizes Tbx1/10 to promote cardiac versus pharyngeal muscle fate in the Ascidian second heart field. PLoS Biol 11:e1001725

Weintraub H, Davis R, Tapscott S, Thayer M, Krause M, Benezra R, Blackwell TK, Turner D, Rupp R, Hollenberg S et al (1991) The myoD gene family: nodal point during specification of the muscle cell lineage. Science 251:761–766

Yamagishi H, Srivastava D (2003) Unraveling the genetic and developmental mysteries of 22q11 deletion syndrome. Trends Mol Med 9:383–389

Zammit PS, Partridge TA, Yablonka-Reuveni Z (2006) The skeletal muscle satellite cell: the stem cell that came in from the cold. J Histochem Cytochem 54:1177–1191

The Lateral Plate Mesoderm: A Novel Source of Skeletal Muscle

Qin Pu, Ketan Patel, and Ruijin Huang

Abstract It has been established in the last century that the skeletal muscle cells of vertebrates originate from the paraxial mesoderm. However, recently the lateral plate mesoderm has been identified as a novel source of the skeletal muscle. The branchiomeric muscles, such as masticatory and facial muscles, receive muscle progenitor cells from both the cranial paraxial mesoderm and lateral plate mesoderm. At the occipital level, the lateral plate mesoderm is the sole source of the muscle progenitors of the dorsolateral neck muscle, such as trapezius and sternocleidomastoideus in mammals and cucullaris in birds. The lateral plate mesoderm requires a longer time for generating skeletal muscle cells than the somites. The myogenesis of the lateral plate is determined early, but not cell autonomously and requires local signals. Lateral plate myogenesis is regulated by mechanisms controlling the cranial myogenesis. The connective tissue of the lateral plate-derived muscle is formed by the cranial neural crest. Although the cranial neural crest cells do not control the early myogenesis, they regulate the patterning of the branchiomeric muscles and the cucullaris muscle. Although satellite cells derived from the cranial lateral plate show distinct properties from those of the trunk, they can respond to local signals and generate myofibers for injured muscles in the limbs. In this review, we key feature in detail the muscle forming properties of the lateral plate mesoderm and propose models of how the myogenic fate may have arisen.

Q. Pu
Department of Anatomy and Molecular Embryology, Institute of Anatomy, Ruhr-University Bochum, Bochum, Germany
e-mail: pujinshuo@hotmail.com

K. Patel
School Biological Sciences, University of Reading, Reading, UK
e-mail: ketan.patel@reading.ac.uk

R. Huang (✉)
Institute of Anatomy, University of Bonn, Bonn, Germany
e-mail: ruijin.huang@uni-bonn.de

© Springer-Verlag Berlin Heidelberg 2015

B. Brand-Saberi (ed.), *Vertebrate Myogenesis*, Results and Problems in Cell Differentiation 56, DOI 10.1007/978-3-662-44608-9_7

1 Introduction

Vertebrates have three sets of skeletal muscles: (1) axial muscles, which facilitate movement of the vertebral column and the skull, (2) the limb and shoulder girdle muscles, which operate the movement of the extremity, and (3) the head muscles, which execute the movement complex for the eye, mouth, tongue, and larynx in the head. According to the classical view, the skeletal muscle cells have two sources: the paraxial and the pre-chordal mesodermal (Table 1). In the trunk and limb, muscle cells originate from the somites, the segmental units of the paraxial mesoderm. The head muscles are derived from the unsegmented cranial paraxial mesoderm (CPM) and the prechordal mesoderm. Recently, skeletal muscle cells have been shown to be derived from the lateral plate mesoderm (LPM). In this review, we concentrate on the myogenesis in the LPM in vertebrates. Recent findings resulting mainly from the chick and mouse models are discussed.

We will first briefly describe the morphogenesis of the somatopleura and the splanchnopleura of the LPM. Then we will discuss the specification and regionalisation of the LPM. In the third part, we will refer the recent studies about the origin of skeletal muscle cells from the LPM. This is followed by the cellular and molecular regulation of their myogenesis. Finally, we will discuss the satellite cells from the LPM.

Table 1 Origin of skeletal muscles

Skeletal muscles		Origin	References
Head	Extra-ocular muscles	Prechordal plate, Cranial paraxial mesoderm	Wachtler et al. (1984) Wachtler and Jacob (1986) Evans and Noden (2006)
	Masticatory and facial muscles	Cranial paraxial mesoderm Cranial lateral splanchnic mesoderm	Noden (1983a), Couly et al. (1992, 1993), Evans and Noden (2006), Nathan et al. (2008), Harel et al. (2009)
	Tongue and infrahyal muscles	Occipital somites	Noden (1983a), Couly et al. (1992), Huang et al. (1999)
Neck	Deep neck muscles	Somites	Huang et al. (2000)
	Dorsolateral neck muscles	Lateral plate mesoderm	Theis et al. (2010)
Trunk	Body wall muscles, Limb muscles Diaphragm muscle Cloacal muscle	Somites	Christ and Ordahl (1995), Christ et al. (1977), Chevallier et al. (1977), Valasek et al. (2005), Bladt et al. (1995)

2 Formation of the Lateral Plate Mesoderm

During gastrulation, cells delaminate from the node and primitive streak and migrate into the space between the epiblast and hypoblast to form the middle germ layer, the mesoderm. The mesoderm compartmentalizes into an axial part (the prechordal mesoderm and the notochord), a paraxial, an intermediate, and a LPM (Selleck and Stern 1991; Psychoyos and Stern 1996; reviewed in Schoenwolf and Alvarez 1992; Schoenwolf et al. 1992). The paraxial mesoderm flanks the axial structure, the notochord, and the neural tube (Christ and Ordahl 1995). It can be subdivided into a pre- and post-otic portion. The pre-otic portion of the paraxial mesoderm termed in many references as cranial paraxial mesoderm (CPM) never undergoes segmentation, while its post-otic part forms segmental units, the somites. Somites are formed by primary segmentation and epithelialisation. The LPM is made of one layer of mesenchymal cells at early stages and then subdivided into a dorsal somatic (SoM) and a ventral splanchnic mesoderm (SpM). The SoM is also called the somatopleure and SpM the splanchnopleure. The subdivision of the LPM starts in the anterior-most region and progresses along the head-to-tail axis towards the caudal end of the embryo (Funayama et al. 1999). In chick embryos, for instance, the LPM is clearly subdivided into two layers at the cephalic level at Hamburger-Hamilton-stage 8 (HH-8). However, the subdivision proceeds only partly at the prospective otic level at the same stage. It furthermore remains one layer at the first somite level. The lateral plate at the first somite level becomes two layers after HH-stage 10 (Fig. 1). The formation of the SoM and SpM is accompanied by the appearance of a coelomic cavity. It appears first in the lateral part of the lateral plate and extends from lateral to medial. The formation of the coelom is controlled by the ectoderm (Funayama et al. 1999).

The intermediate mesoderm is not formed at the head level and lies posterior to the cervical somite level. Due to the lacking of the intermediate mesoderm, the CPM and the occipital somites are continuous with the LPM. The boundary between them is difficult to identify morphologically. It can be visualised only by genetic markers. For instance, *Pax3* marks only the somites, but not the LPM (Theis et al. 2010). In chick embryos, *Alx4, Cyp26c1*, and *Twist* are expressed in CPM and occipital somites (Nathan et al. 2008; Bothe and Dietrich 2006; Tirosh-Finkel et al. 2006; Dastjerdi et al. 2007). The LPM is characterised by the expression of *FoxF1*, a forkhead box F1 transcription factor, and of *Hand1* and *Hand2* expression (Srivastava et al. 1995; Charite et al. 2000; Yelon et al. 2000; Deimling and Drysdale 2009). After the subdivision, the *FoxF1* expression becomes restricted to the ventral splanchnic layer, while the *Irx3* (Iroquois class homeodomain transcription factor) expression appears in the dorsal somatic layer (Funayama et al. 1999; Mahlapuu et al. 2001). Expression of a set of second heart field genes is found also in the SpM of chick embryos at HH-8 (Nathan et al. 2008).

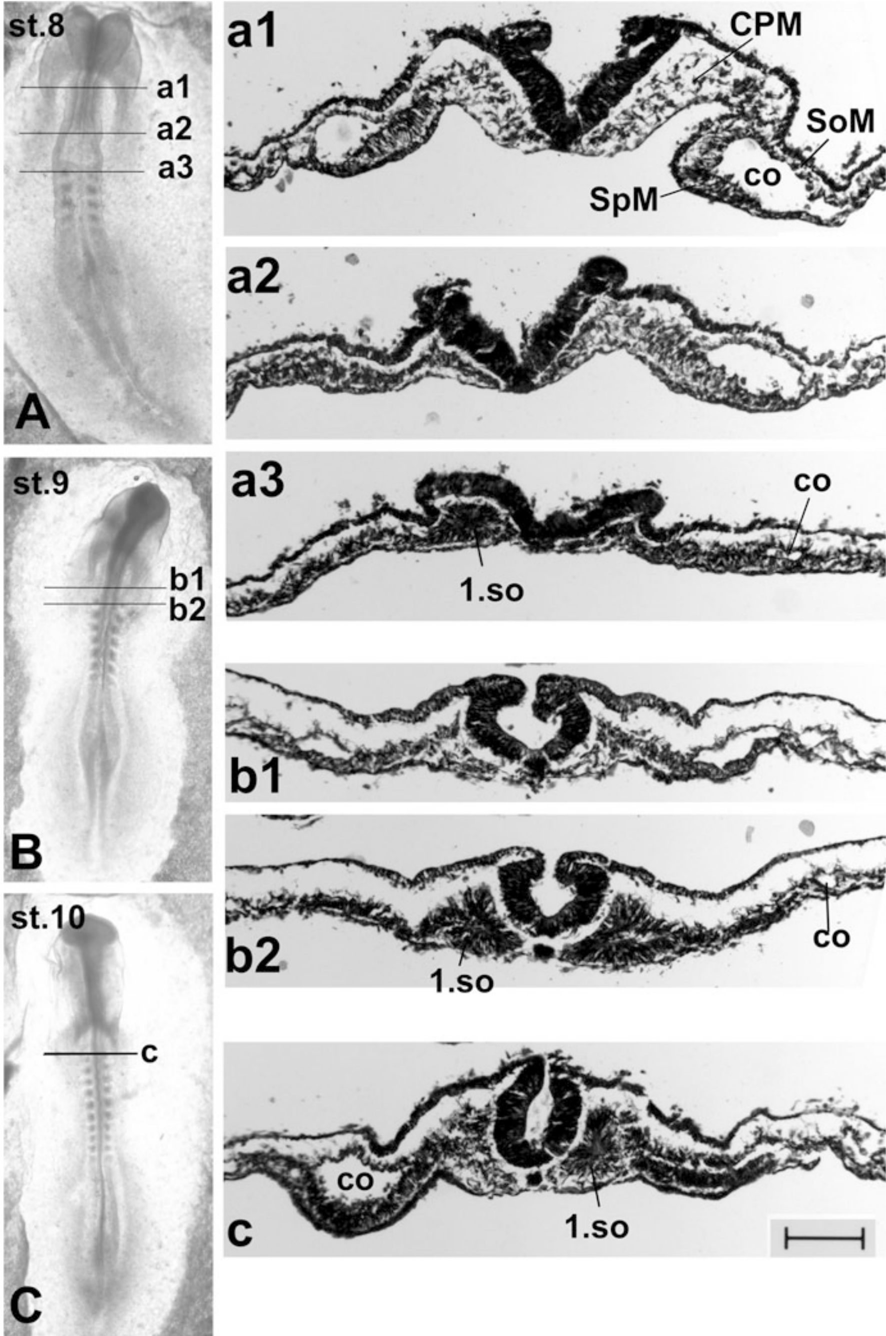


Fig. 1 Formation of the somatopleure and splanchnopleure from the lateral plate mesoderm proceeds gradually in cranial to caudal direction. (**A**) Dorsal view of a chick embryo at Hamilton and Hamburger-stage 8 (HH-8). Four somites are formed. (**a1**) Transverse section at the cranial cephalic level indicated by line a1 in Fig. A. The neural tube (nt) is still not yet closed. The cranial

3 Specification of the Lateral Plate Mesoderm

According to the developmental properties, the lateral plate can be subdivided into the anterior and the posterior LPM (Waxman et al. 2008; Zhao et al. 2009). The boundary of these two mesoderm regions lies at the level of somite 5–6 in mouse embryos (Waxman et al. 2008). While *Hand1* and *Hand2* genes are expressed throughout the entire LPM, *NKx2.5* and *Tbx20* expression is restricted to the anterior LPM (Buchberger et al. 1996; Kraus et al. 2001; Yamagishi et al. 2004; Deimling and Drysdale 2009). The pharyngeal LPM is characterised by the *Tbx1* expression (Garg et al. 2001).

The anterior LPM contributes to the heart formation and is considered as cardiac mesoderm. Firstly, myocardial progenitor cells populate a region of the lateral plate on either side of the neural folds. This region is considered as the primary heart field. A primary heart tube forms from each side of the primary heart field. The bilateral symmetrical heart tubes fuse into one heart tube, consisting of a venous and an arterial pole. As development proceeds, further myocardial progenitor cells are recruited at the both poles of the heart tube. The progenitor cells for additional growth of the arterial pole, which gives rise to the outflow tract and right ventricle, was recently shown to arise from the SpM of the pharyngeal LPM known as the secondary or anterior heart field (SHF/AHF).

The entire posterior LPM at early stages of development has been reported to have limb-forming potential. Stephens et al. (1989) explanted lateral plate with overlying ectoderm and underlying endoderm from the neck (somites 10–14), wing (somites 15–20), flank somites 21–25), and leg (somite 26-end of the embryo) of

Fig. 1 (continued) paraxial mesoderm (CPM) is composed of loosely arranged mesenchymal cells. The somatic mesoderm (SoM) and splanchnic mesoderm (SpM) are formed in the lateral plate mesoderm. A coelomic cavity (co) is surrounded by these two cell layers. The SpM is made of cylindrical epithelial cells, while the cells in the SoM layer are flat. (**a2**) Transverse section through the level indicated by line a2. The lateral plate mesoderm is presented as a layer of cells on the left side. In contrast, the right lateral plate contains a dorsal and ventral layer, the SoM and SpM, surrounding a coelom (co). (**a3**) Transverse section through the first somite (1.so) level indicated by line a3 in the Fig. A. The wide neural plate lies on the somite. While the section is located at the level of the middle part of the first somite on the left side, the section is cut through the cranial edge of the right first somite. Only a small coelom (co) is present on the right side. (**B**) Dorsal view of a HH-9 chick embryo. Seven somites are formed. (**b1**) Transverse section through the cephalic level indicated by line b1 in the Fig. B. The neural tube is starting to close. A coelom can be seen on both sides in the lateral part of the lateral plate mesoderm. (**b2**) Transverse section through the first somite (1.so). Only a very small coelom can be seen in the most lateral part of the lateral plate mesoderm. The main part of the lateral plate has not yet been subdivided into a somatic and a splanchnic mesoderm. (**C**) Dorsal view of a HH-10 embryo with 10 somites. (**c**) Transverse section through the first somite (1.so) on the right side and the unsegmented CPM level on the left side. At this level, the neural tube is closed and the lateral plate mesoderm is differentiated into a dorsal somatic (SoM) and a ventral splanchnic mesoderm (SpM). The coelom on the left side is located more cranially and has advanced in development compared to the right side. *Bar*: 100 μm for the sections

stage 11–14 chick embryos. Each explant was allowed to grow in the coelom of 2.5–3 day-old-embryo for further 7–8 days. All explants formed limb structures. This means that the limb field is present throughout the posterior LPM.

Our previous studies show that the skeletal muscle forming potential is restricted to the anterior LPM. Only the lateral plate at the level of somites 1–3 (occipital region) contributes to cucullaris muscle in chick embryos (Theis et al. 2010). The lateral plate tissue which was grafted from the neck region into the occipital region could not form skeletal muscles. This indicates that the cervical lateral plate has no intrinsic myogenic potential and cannot be induced to form muscles through local cues in the occipital region. After the occipital LPM was grafted into the limb level, it could not form skeletal muscle cells. This indicates that the skeletal muscle forming potential of the cranial LPM is not cell-autonomous and its myogenesis requires local inductive signals.

4 Origin of Skeletal Muscle Cells from the Lateral Plate Mesoderm

The skeletomyogenic potential of the LPM was first observed by means of cell lineage tracing in mouse embryos. The murine *myocyte enhancer factor-2C (MEF-2C)* has been shown to be expressed in the secondary heart field and controls the heart looping and right ventricular chamber formation (Lin et al. 1997; Dodou et al. 2004). Verzi et al. (2005) used the *mef2c*, an anterior heart field promoter and enhancer, to direct the expression of cre recombinase exclusively in the anterior heart field. The Cre expression was reported by Cre-dependent lacZ activity (Soriano 1999). They showed that the mef2c–AHF–Cre transgene expression overlaps with markers of the secondary/anterior heart field. As development proceeds, not only the outflow tract and right ventricle but also the mesodermal component of the branchial arches are marked by the activity of the mef2c-AHF-Cre transgene (Verzi et al. 2005). It can be assumed that progenitor cells from the secondary heart field may migrate into the branchial arch to form skeletal head muscles.

This assumption was strengthened by the study using Isl1-Cre mice (Nathan et al. 2008). Isl1 is first expressed in the cranial splanchnic mesoderm, especially in the secondary heart field (Cai et al. 2003). During further development, *Isl1*+ cells are found in the mesenchymal core of both 1st and 2nd branchial arch. Finally, *Isl1*+ cells are identified in muscles derived from these both branchial arches, such as the mylohyoid, styloid, digastric, buccinator, and facial subcutaneous muscles. This observation predicts that the head muscle and cardiac muscle share a common cell lineage. This is confirmed by a retrospective clonal assay that cells derived from a single precursor are found in both branchiomeric head muscles and right ventricular and arterial pole myocardium (Lescroart et al. 2010).

The DiI labelling experiment in the chick confirmed the observation of the cre-lineage tracing experiment in the mouse (Nathan et al. 2008). After DiI was

injected into the cranial SpM, cells of the outflow tract and the first branchial arch were labelled. Because cells from the CPM also migrate into the branchial arch to form head muscles (Noden et al. 1999; Tirosh-Finkel et al. 2006), the topographic relationship of the CPM and SpM descendants in the branchial arch were addressed. By means of injection of DiI and DiO into the CPM and SpM, respectively, of the same embryo, Nathan et al. (2008) showed that the CPM-derived precursor cells are located in the proximal part of the first branchial arch, while the SpM-derived cells reside in the distal part of the first branchial arch. Due to dilution of dye after several cell divisions, the cell fate of the precursor cells in the CPM and SpM cannot be followed directly. So Nathan et al. (2008) labelled cells in the proximal and distal mesenchymal core of the first branchial arch using DiI and DiO, respectively. Although neural crest cells in the mesenchymal core of the branchial arch were also labelled, these cells are known to never differentiate into myocytes. The dye labelled proximal myogenic population was found to contribute to the masseter muscle, while the distal myogenic population gives rise to the intermandibular muscles. The contribution of the CPM to the mastication muscle was confirmed by the retrospective clonal assay made by Lescroart et al. (2010), who observed that a cell lineage which gave temporalis and masseter muscle provided also muscle cells for the extraocular muscle.

A further LPM-derived muscle is a dorsolateral neck muscle, the cucullaris muscle in birds, corresponding to the trapezius and sternocleidomastoideus in mammals. The M. cucullaris in birds is a very broad and flat muscle, which can be subdivided into M. cucullaris capitis and M. cucullaris cervicis. The cucullaris capitis muscle has its origin in the lateral surface of the head, Os squamosum (a part of the temporal bone) and extends caudally to the neck. The muscle sheets of both sides touch each other in the dorsal region of the neck from the 2. to the 7. cervical segments, forming a hood. Then, the muscle is subdivided into three portions. The Pars interscapularis sends muscle fibres into the skin in front of the shoulder joint. The Pars propatagialis consists of a few muscle fibres, which reach the flight skin (propatagium). The Pars clavicularis draws ventrally over the crop and extends as a thin and triangular muscle sheet between the furcula, the forked clavicle bones. The terminal tendon of this muscle touches finally the rostrum sterni. The M. cucullaris capitis has its attachment in the shoulder girdle region. The M. cucullaris cervicis encompasses the caudal part of the neck and the shoulder region.

In our previous study, we demonstrated that this muscle originates from the LPM at the level of the occipital somites (Theis et al. 2010). To trace the LPM descendants, we replaced a piece of the LPM adjacent to the three first occipital somites of a host chick embryo with the same tissue part from either quail or transgenic chick embryos expressing cytoplasmic GFP under control of the beta-actin promoter (provided by Dr. H. Sang). While quail cells can be identified using a perinuclear antibody only on tissue sections, GFP cells can be seen in whole mounts of embryos. So we observed that GFP cells distributed in the neck region according to the same pattern as the cucullaris muscle. GFP cells extended from the head to the shoulder region. Furthermore, GFP cells populate also in the upper back region,

corresponding to the trapezius muscle. In sections, GFP cells as well as quail cells were identified as muscle cells in the M. cucullaris.

In previous studies, it was reported that the cucullaris muscle is composed of myoblasts from somites in chicken (Noden 1983a, b, 1986a, b; Noden et al. 1999; Couly et al. 1993; Huang et al. 1997, 2000). The somitic origin of the cucullaris muscle was confirmed by a study in Ambystoma mexicanum embryos (Piekarski and Olsson 2007). They injected FITC-dextran into cranial somites and observed FITC-labelled cells in this muscle. In view of numerous studies evidencing the somitic contribution to the cucullaris, two scenarios were proposed. First, the cucullaris muscle could derive muscle progenitors from both somites and LPM, in a manner similar to the branchiomeric musculature (Harel et al. 2009). Second, the cucullaris muscle is derived only from one of these structures, as experiments leading to the aforementioned conclusion could have arisen due to tissue contamination during the transplantation. We improved the transplantation procedure by using dispase I to reduce the tissue contamination and quantified the cellular contribution of the somite and the lateral plate. We found that the somitic contribution was quite minor, whereas there was a very high density of tissue originating from the lateral plate in the cucullaris muscle. These results demonstrate that the cucullaris muscle is mainly derived from the LPM.

Our genetic cell lineage tracing study in mouse exclude the somitic contribution to the cucullaris muscle and substantiate the finding from the transplantation experiment in birds (Theis et al. 2010). In mammals, the trapezius and sternocleidomastoideus muscle are avian homologues of the cucullaris muscle. Both neural crest cells and somite cells express *Pax3* (Goulding et al. 1991, 1993; Goulding and Paquette 1994). In $Pax3^{Cre}$:$Rosa^{STOP/YFP}$ embryos, these cells express Cre-recombinase under the endogenous *Pax3* promoter, which ultimately initiates YFP expression from a floxed Rosa allele. YFP fluorescence marks all cells with a past or present history of *Pax3* expression. We found YFP activity in most trunk muscles. However, we could not find YFP-positive cells in the muscle fibres of the trapezius and sternocleidomastoideus as well as other head muscles. YFP cells could be found only between the muscle fibres in the head. These were the neural crest-derived cells which form connective tissue of these muscles (Noden 1983a). These results confirm the sole contribution of the LPM to the cucullaris muscle in birds and its homologues in mammals.

Taken together, the LPM participates in the formation of branchiomeric muscles and dorsolateral neck muscles. While branchiomeric muscles are comprised of myoblasts from both CPM and LPM, the dorsolateral neck muscle is derived only from the lateral plate.

5 Lateral Plate-Derived Muscles Differentiate Later Than Other Muscles

After examining the formation of the skeletal musculature in head and trunk, we found that the cucullaris muscle developed in chick embryos very late compared to other skeletal muscles (Theis et al. 2010). First, the cucullaris myogenic cells require a long period to reach their destination. The cranial to caudal migration of the LPM cells from the level of somite 1-3 is detectable first by HH-14. By HH-20, the transplanted cells extended just to the sixth somite level. The caudal end of the grafted tissue reached the anterior limb base by HH-26. The morphological form resembling the adult muscle was achieved by HH-30.

The differentiation of the cucullaris muscle also occurs at a relatively late stage. MyoD expression was found to be initiated in somites, limb, and branchial arches at HH-24. The first faint expression of *MyoD* was detected in the cucullaris muscle at HH-26. Just after HH-30, *MyoD* had reached its entire extent of the muscle. Correspondingly, the terminal muscle markers could be detectable in somites, extremities, and heart at HH-24. Differentiated myoblasts could be seen in the second and third branchial arch at HH-26. However, the myoblasts were detected in the cucullaris muscle at HH-30.

The late differentiation of the cucullaris muscle predicts that the myogenic precursor required a longer period for proliferation than other muscles. The possible reason might be that the muscle is very long and large. So the progenitor cells require long time for generating a large pool of myogenic cells. It is still unknown how the cell proliferation is controlled in this process.

This feature of late development is conserved in vertebrates. For instance, in the turtle at stage 15, differentiated muscle was found in the head, trunk and limb with exception of the cucullaris muscle. The cucullaris muscle was clearly discernible at stage 17. It means that also in the turtle, the cucullaris develops later than the other muscles.

6 Molecular Regulation of the Myogenesis in the Lateral Plate Mesoderm

Although there are only few studies concerning the LPM myogenesis directly (Theis et al. 2010; Harel et al. 2009; Nathan et al. 2008; Dong et al. 2006; Verzi et al. 2005), numerous studies investigating head myogenesis provide some knowledge about myogenesis in the LPM (Harel et al. 2009; Sambasivan et al. 2009; Ericsson and Olsson 2004; Ericsson et al. 2004; Olsson et al. 2001; Bothe and Dietrich 2006; Rinon et al. 2007; Lescroart et al. 2010; Couly et al. 1992; Noden 1983a, b; Noden 1986a, b; Marcucio and Noden 1999; Noden et al. 1999; reviewed by Noden and Francis-West 2006; Tzahor 2009; Sambasivan et al. 2011; Buckingham and Vincent 2009; Buckingham et al. 2005; Kelly et al. 2004).

During the embryonic myogenesis in somites, *Pax3* and *Pax7* are expressed the dermomyotome and play pivotal roles for the cell proliferation and survival of the somitic myogenic progenitors (Relaix et al. 2005; Sambasivan and Tajbakhsh 2007). *Pax3* and *Pax7* act upstream of *MyoD* (Tajbakhsh et al. 1997). However, both these transcription factors are not involved in the myogenesis in the cranial mesoderm (Noden and Francis-West 2006). The cranial myogenesis is regulated by a series of different transcription factors, such as *Pitx2, Tbx1, MyoR, Capsulin, and Isl1* (reviewed by Sambasivan et al. 2011; Tzahor 2009).

Pituitary homeobox 2 (*Pitx2*), a paired-related homeobox gene, is expressed in the cranial mesoderm including the periocular mesenchyme and the core mesoderm of the first branchial arch (Gage et al. 1999; Kitamura et al. 1999). In *Pitx2*-null embryo, EOMs are missing (Gage et al. 1999; Kitamura et al. 1999). *Pitx2* is required for MyoR expression in the first branchial arch muscle precursors. In the *Pitx2* null mouse, cells derived from the SpM, which were marked with Mef2c-AHF-Cre LacZ expression (Verzi et al. 2005), could not migrate into the first branchial arch (Dong et al. 2006). *Pitx2* regulates specifically the early muscle specification of the first branchial arch (Shih et al. 2007a, b).

The T-box containing transcription factor Tbx1 plays a critical role in the head muscle and cardiac out flow tract development (Kelly et al. 2004). Tbx1 is required for the activation of *Myf5* and *MyoD* in all branchiomeric muscles including both CPM- and LPM-derived muscles, whereas Tbx1 is not involved in the regulation of extraocular and tongue muscles. In the *Tbx1*-mutant, the formation of branchiomeric muscles such as jaw, craniofacial and laryngeal muscles, as well as trapezius are affected.

MyoR (Msc, musculin) and Capsulin (Tcf21) are bHLH transcription factors expressed in the head and body muscles. Both are postulated to repress the myogenic differentiation. Mutations of these both genes lead to the absence of a subset first arch-derived jaw muscles (masseter, pterygoid, and temporalis muscles). However, distal muscles of the first branchial arch (anterior digastri and mylohyoid) were not affected (Lu et al. 2002). This suggests that MyoR and Capsulin specifically control the formation of the CPM-derived but not the SpM-derived muscles in the first branchial arch.

Isl1 (the LIM homeodomain protein Islet1) plays pivotal role for the proliferation, differentiation and lineage specification of distinct cardiovascular precursors (Cai et al. 2003; Laugwitz et al. 2005; Moretti et al. 2006). *Isl1* is expressed also in the branchial muscle progenitors derived from the SpM (Nathan et al. 2008). After overexpression of *Isl1* by means of RCAS-Isl1 in chick embryos, *MyoD, Myogenin*, and *MyHC* were blocked in CPM explants *in vitro* and in the first branchial arch in vivo (Harel et al. 2009). Since *Isl1* expression could be induced by BMP4 which has been shown to inhibit myogenesis in both somites and head mesoderm (Tirosh-Finkel et al. 2006), one can assume that BMP4 may fulfil its inhibitory function on myogenesis via inducing *Isl1* expression (Harel et al. 2009). Furthermore, *Isl1* was inhibited by overexpression using electroporation of Wnt3-IRES-GFP into the surface ectoderm. In agreement, inhibition of Wnt pathway with sFrp2 and sFrp3 resulted in an expansion of *Isl1* expression (Nathan et al. 2008).

As described above, the cucullaris muscle in birds represents a pure lateral plate-derived muscle (Theis et al. 2010). We showed that the trunk myogenic programme is not involved in the development of this muscle. *Pax3* and *Pax7* which drive somite myogenic progenitor cell proliferation while suppressing differentiation (Amthor et al. 1999; Amthor et al. 1998) were never expressed in this muscle in chick embryos. Instead of expressing *Pax3* and *Pax7*, genes of the head myogenic programme, such as *MyoR, Tbx1,* and *Capsulin* were expressed in the anlagen of the cucullaris muscle during the early development in chick embryos. The role of the head myogenic programme in the development of the cucullaris muscle was confirmed by examining $Pax3^{cre}$:$Rosa^{stop/YFP}$, $Pax3^{sp/sp}$:$Myf5^{nlacZ/nlacZ}$, and $Tbx1^{-/-}$ mouse mutants (Engleka et al. 2005; Kelly et al. 2004). We found that myoblasts of the trapezius and sternocleidomastoid, the cucullaris homologues in mammals, never expressed Pax3 . Furthermore, the trapezius and sternocleidomastoid muscles were present in the $Pax3^{sp/sp}$:$Myf5^{nlacZ/nlacZ}$mutants, in which all somite-derived muscles were missing. This is concordant with the finding in the $Tbx1^{-/-}$ line, which failed to form both of these neck muscles. Additionally the trapezius has a molecular history for *Isl1*, which might repress the differentiation and promote the proliferation of the myogenic progenitors during the early development of head muscles (Harel et al. 2009). Our lineage tracing experiment suggests that these muscles require a very long period for generating enough number of myoblasts to form a large muscle.

The cucullaris muscle is a long muscle sheet which is located from the occipital to the thorax region. It is still unknown how myogenic cells originating from the occipital region reach the shoulder and thorax region. Recently, myotomal cells have been shown to extend from one segment to the next segment (Chankiewitz et al. 2014). This myotomal extension is controlled by a thymosin beta 15-like peptide. Myogenic cells of the cucullaris might extend from their origin to the thorax and shoulder region by means of the same mechanism.

7 Cranial Neural Crest Cells Form the Connective Tissue of the Lateral Plate-Derived Muscle

Cranial neural crest (CNC) provides a wider range of differentiation potential than trunk crest. Their derivatives include not only neurons, glia, and pigment cells, but also skeletal cells of the head. In addition, CNC has also been reported to form the connective tissue of branchiomeric muscles (Noden 1983a; Kontges and Lumsden 1996). Using the quail-chick cell lineage tracing technique, in which the CPM and cranial neural crest from quail to chick was transplanted, Noden (1983a) observed that the branchiomeric muscle has two components: myogenic cells are of CPM origin and the connective tissue is derived from the cranial neural crest. Using the same tracing technique, Kontges and Lumsden (1996) mapped the neural crest (NC) subpopulations of individual rhombomeres. They observed that each

rhombomeric NC population forms both the connective tissues of specific branchiomeric and hypoglossal muscles and their attachment sites on the mandibular and lower jaw skeleton.

This relationship between neural crest and paraxial mesoderm was extended to the neck region (Matsuoka et al. 2005). The vertebrate neck is a mobile interconnection of the head and trunk (McGonnell 2001). The length of the neck varies from one segment to 76 segments. The primitive amphibians were in possession of the first cervical vertebra (Torrey 1978). The fossil diapsid Muraenosaurus had 76 cervical vertebrae (Young 1981). While mammals have 7 cervical vertebrae, the avian cervical spinal column contains 13 (pigeon) to 25 segments (swans) (Burke et al. 1995). In spite of the different length of the neck, the dorsal and ventral shoulder muscles can extend from head to trunk, operating the shoulder girdle. Using cre-recombinase-mediated Wnt1 and Sox10 transgenesis, Matsuoka et al. (2005) mapped the long-term cell fate of NC in mouse embryos. They revealed that the connective tissue of dorsal and ventral neck muscles at both head and shoulder attachment sites is of NC origin. This was confirmed by other research groups who used also the Wnt1 transgenic (Theis et al. 2010; Valasek et al. 2010). We further investigated the originating axial level of the NC in our previous study. Using quail–chick chimaeras, we found that the neural crest cells formed the cucullaris muscle connective tissue, the dorsal neck muscle in birds, are derived from the occipital level (Theis et al. 2010). This observation led us to predict that both NC-derived connective and LPM-derived myogenic precursor cells originate from the same axial level, the occipital level, and migrate from the head region caudally to the trunk during formation of the neck.

The contribution of cranial neural crest cells to the connective tissue of cranial muscles was investigated also in amphibians (reviewed in Schmidt et al. 2013 and Ericsson et al. 2013). Using DiI labelling, green fluorescent protein (GFP) mRNA injection and transplantation of neural folds, Olsson's group showed that cranial neural crest cells form the connective tissue but not the myofibers in the branchiomeric muscles in Bombina orientali (Olsson et al. 2001) and in Ambystoma mexicanum (Ericsson et al. 2004).

8 Cranial Neural Crest Cells Determine the Patterning of the Lateral Plate-Derived Muscle

The CNC origin of the connective tissue suggests an important function of the neural crest in the patterning of the LPM-derived muscle. Following heterotopic transplantation in which neural crest at the level of the presumptive first branchial arch was grafted to the level of the presumptive second and third branchial arch, grafted cells form a duplicated first branchial arch skeletal system in the ectopic location (Noden 1983b). Furthermore, the pattern of the branchiomeric muscle is dependent upon properties of the grafted neural crest. These results indicate that

neural crest cells are prespecified prior to their migration into branchial arch regarding the patterning of the branchiomeric skeletal system and the form of the associated muscle. The patterning information of the neural crest is not only axial level specific but also species specific. Homotopic transplantation of neural crest from a duck into a quail embryo led to the formation of duck-specific beak in the quail host (Tucker and Lumsden 2004).

After the migration into the branchial arch, CNC cells and the muscle precursor cells are arranged in a highly organised fashion. The muscle progenitors are located in the core of the branchial arch, while CNC cells are located beneath the ectoderm. Hence, the CNC cells enclose the muscle precursor cells and separate them from the overlying surface ectoderm (Noden and Trainor 2005). The neural crest cells streaming into a given branchial arch form both attachment sites of the muscle which is derived from the same branchial arch. Thus, the connective tissue forming neural crest cells and the myogenic cells take the same migratory route. Thereby, the neural crest cells could provide guidance cues for the migratory myogenic progenitors (Kontges and Lumsden 1996; Olsson et al. 2001; Matsuoka et al. 2005).

Based on the observation that surgical removal of the neural crest did not interrupt the early myogenesis in the branchial arch, the early branchiomeric myogenesis is independent of the neural crest cells (Olsson et al. 2001; Tzahor et al. 2003; Ericsson et al. 2004; Ericsson and Olsson 2004; Rinon et al. 2007). In amphibian embryos, myogenesis is initiated in absence of neural crest cells. However, the myogenic progenitor cells cannot reach their destinations (Olsson et al. 2001; Ericsson et al. 2004; Ericsson and Olsson 2004). In chick embryos, *Myf5, MyoD, Tbx1* and *capsulin* were expressed in the branchial arches after neural crest ablation. However, their expression pattern was interrupted (Tzahor et al. 2003; Rinon et al. 2007). These findings indicate that neural crest cells are essential for the pattern of myogenic gene expression. They are, however, dispensable for the initiation of the myogenesis.

The results from the surgical removal in avian and amphibian embryos might be influenced by the manipulation limitation and thus their interpretation could be very complex, since neural crest is known to regenerate following ablation (Saldivar et al. 1997; Scherson et al. 1993; Vaglia and Hall 1999). To avoid the problem with post-operative regeneration of neural crest in chick and amphibian embryos, Rinon et al. (2007) analysed mutant mice in which crest cells were genetically ablated. One of such mouse line was the Hoxa1/Hoxb1 double-mutant mouse, in which crest cells fail to migrate into the second branchial arch. Though early muscle markers, such as *capsulin* and *Tbx1*, were detected in the second branchial arch, their expression was broader in the mutant than those in the control mouse.

9 Lateral Plate Mesoderm and Muscle Stem Cells

The satellite cell located in the basal lamina of a muscle fibre is the resident stem cell of skeletal muscle, carrying out the routine maintenance, hypertrophy, and repair of damaged adult skeletal muscles (Mauro 1961; Buckingham 2007; Kuang et al. 2007; Zammit et al. 2006). Satellite cells maintain a stable stem cell pool by means of the primary self-renewal mechanism (Collins et al. 2005; Montarras et al. 2005; Sacco et al. 2008). *Pax7* expressed by satellite cells controls the generation of embryonic myogenic precursor cells (Seale et al. 2000; Lepper et al. 2009). In double mutants of *Pax3* and *Pax7*, muscle development was severely affected (Relaix et al. 2005). Once activated, satellite cells co-express *Pax7* with *MyoD*. After the cell division, one of the daughter cell down-regulates *Pax7* and maintains *MyoD*. As a result it induces myogenin and differentiates into myoblast. The other daughter cell down-regulates *MyoD* and maintains *Pax7* and remains in a quiescent state (Halevy et al. 2004; Zammit et al. 2004).

In vertebrates, trunk and limb skeletal muscles originate from somites, segmented paraxial mesodermal structures (Christ and Ordahl 1995). Using the quail–chick cell lineage tracing system, Armand et al. (1983) reported for the first time the embryonic origin of the satellite cells of the trunk muscle from the somites. During the development, the somite undergoes a dorsoventral compartmentalisation, resulting in a ventral mesenchymal sclerotome and a dorsal epithelial dermomyotome. While the sclerotome is responsible for the formation of the axial skeleton, the dermomyotome gives rise to dermal and muscular tissues (Stockdale et al. 2000). Gros et al. (2005) and Relaix et al. (2005) demonstrated that the dermomyotome is also the source of satellite cells. In chick embryos, different somitic compartments were labelled by electroporation of GFP-vectors and by quail–chick chimaeras. Using these two complementary cell tracing techniques, Gros et al. (2005) concluded that the central part of the dermomyotome contributes to both embryonic myogenic precursors and adult satellite cells. In mice, cells of the central dermomyotome labelled by reporter genes targeted into Pax3 and Pax7 loci were found to delaminate and migrate into the early myotome. In the late development, these cells were found to integrate into the adult muscle as satellite cells (Relaix et al. 2005). The limb muscle satellite cells originate from the ventral dermomyotome which provides hypaxial muscle precursors for the limb and ventral body wall (Schienda et al. 2006).

In the head, branchiomeric muscle satellite cells were found to have two sources. Using quail–chick chimaeras, Harel et al. (2009) identified CPM-derived cells in the position of satellite cells in masticatory muscles. They found that 90 % of satellite cells of eye and masticatory muscles were of CPM origin. *Islet1* is expressed in the splanchnic layer of the LPM. In the *Islet1Cre* mouse line, Islet1$^{+/+}$ cells were found to contribute to 90 % satellite cells of masseter and anterior digastric muscles. It is hard to understand how 90 % of satellite cells of a muscle are of CPM origin in chick embryos and the same muscle receives 90 % satellite cells from the SpM in mouse. The reason might lie in the difference

between species. The reason for this finding is possibly the continuum from the CPM to the LPM as described above. This could lead to the problem with contamination of CPM cells with LPM cells during the tissue transplantation in birds. Furthermore, the overlap expression of *Isl1* in the CPM and SpM may be so strong that *Isl1*-positive cells not only represent cells derived from the SpM, but also the CPM.

Although it remains to be clarified whether the lateral plate at the occipital level gives rise to satellite cells of the dorsolateral neck muscles (cucullaris in birds and trapezius and sternocleidomastoideus in mammals), the above described observations predict that both myogenic and satellite cell progenitors arise from common embryonic origins. They travel along the same route to their destinations and maintain their spatial neighbourhood during development.

There are no studies investigating directly satellite cells being derived from the LPM. Based on the observation that *Isl1*-expressed SpM gave rise to a vast majority of satellite cells of the masseter muscle, results arising from the studies on masseter can be considered as representative for the lateral plate-derived satellite cells (Ono et al. 2010; Harel et al. 2009). After obtaining the percentage of satellite cell number on the total number of nuclei per myofiber, Ono et al. (2010) found that masseter muscle has fewer satellite cells than limb muscle. After counting the percentage of self-renewing satellite cells (Pax7+/MyoD-) and differentiating satellite cells (Pax7-/MyoD+, Pax7-/myogenin +) in relation to the total number of satellite cells per myofiber at different culture time points, they found that masseter-derived satellite cells differentiate later than those from a limb muscle. Furthermore, satellite cells of the masseter muscle have stronger proliferative ability than those from limb muscles. Gene expression profiles of satellite cell-derived myoblasts (after 4 h of isolated satellite cell culture) were compared between limb muscles and masseter muscle by measurements of gene expression intensity using quantitative RT-PCR. The results demonstrate that satellite cell-derived myoblasts maintain their molecular profile from their embryonic origin. *Pax3* was robustly expressed in myoblasts from limb muscles, whereas it was not detectable in myoblasts from masseter. *Pitx2b* and *Pitx2c* were significantly higher in myoblasts from limb muscles than those from masseter. *Pax7* and *Mrf4* were more highly expressed in myoblasts from masseter muscle than in those from limb muscles. It is noteworthy that digastricus muscle-derived satellite cells (solely derived from the lateral plate) displayed a very high amplitude of gene expression of *Nkx2.5* compared to those from limb muscle. Furthermore, masseter-derived cells expressed much stronger *Tcf21* (*Capsulin*), which represents an important transcriptional factor for craniofacial muscle formation (Lu et al. 2002), than those from limb muscles (Ono et al. 2010; Harel et al. 2009).

During embryonic myogenesis, BMP4 induces cardiogenesis, while it blocks myogenesis in both head mesoderm and somites (Tirosh-Finkel et al. 2006; Reshef et al. 1998). BMP4 induces the proliferation of satellite cells derived from both trunk and head muscles. The inhibition of the myogenic differentiation through BMP4 was less potent in satellite cells derived from the masseter muscle than those from limb muscles (Harel et al. 2009). The proliferative state of satellite cells was

revealed by *Myf5* and *Pax7* expression, while their differentiation activity was identified by *MyoG* and *MyHC* and also viewed by myofiber formation. In addition, BMP4 induces stronger expression of *Isl1* and *Tbx20* in satellite cells from masseter muscle than those from limb muscles. *Isl1* and *Tbx20* which are considered as cardiac markers were shown to repress myogenic differentiation in the head myogenesis, up-regulation of *Isl1* by BMP4 predicts that BMP4 maintains the plasticity of satellite cells of head muscles (Harel et al. 2009).

In spite of their different embryonic origin and regulatory properties, lateral plate-derived satellite cells can regenerate somite-derived muscles (Harel et al. 2009; Ono et al. 2010). It has been shown that limb-derived satellite cells transplanted into an irradiated limb muscle can generate hundreds of muscle fibres (Collins et al. 2005). Ono et al. (2010) performed transplantation of satellite cells isolated from the masseter and the extensor digitorum longus (a limb muscle), respectively, into the tibialis anterior muscle (a limb muscle). In the fourth week of posttransplantation, they observed that the amount of donor-derived newly formed muscle fibres was not significantly different between muscles receiving either masseter- or extensor digitorum longus-derived satellite cells. By means of single myofiber transplantation, Harel et al. (2009) obtained similar findings. These findings indicate that independent upon their origin, satellite cells can respond to local signals in the limb to generation myofibers.

10 Perspectives

It is interesting to speculate on the mechanisms that imbued myogenic properties on the anterior lateral plate mesoderm. We discuss here two possibilities that may lead to this outcome. In the first scenario, we propose that the head mesoderm, that has myogenic properties, extends posteriorly adjacent to the first three somites. This is feasible since the head and lateral plate mesoderm are continuous. Alternatively, the occipital lateral plate mesoderm may have been patterned to gain characteristics of head mesoderm by the posterior extension of a molecular boundary that confers myogenic properties. There are numerous examples in the animal kingdom where molecular boundary shifts regulate the development of tissues, both in invertebrates and vertebrates. Further investigation using a combination of cell tracing and molecular analysis of key genes especially members of the Hox family of transcriptional factors will be needed to determine which of these possibilities is responsible for the lateral plate myogenicity.

Acknowledgements This study was supported by the German foundation grant (DFG, Hu729/10).

References

Amthor H, Christ B, Weil M, Patel K (1998) The importance of timing differentiation during limb muscle development. Curr Biol 8:642–652

Amthor H, Christ B, Patel K (1999) A molecular mechanism enabling continuous embryonic muscle growth—a balance between proliferation and differentiation. Development 126:1041–1053

Armand O, Boutineau AM, Mauger A, Pautou MP, Kieny M (1983) Origin of satellite cells in avian skeletal muscles. Arch Anat Microsc Morphol Exp 72:163–181

Bladt F, Riethmacher D, Isenmann S, Aguzzi A, Birchmeier C (1995) Essential role for the c-met receptor in the migration of myogenic precursor cells into the limb bud. Nature 376:768–771. doi:10.1038/376768a0

Bothe I, Dietrich S (2006) The molecular setup of the avian head mesoderm and its implication for craniofacial myogenesis. Dev Dyn 235:2845–2860. doi:10.1002/dvdy.20903

Buchberger A, Pabst O, Brand T, Seidl K, Arnold HH (1996) Chick NKx-2.3 represents a novel family member of vertebrate homologues to the Drosophila homeobox gene tinman: differential expression of cNKx-2.3 and cNKx-2.5 during heart and gut development. Mech Dev 56:151–163

Buckingham M (2007) Skeletal muscle progenitor cells and the role of Pax genes. C R Biol 330:530–533. doi:10.1016/j.crvi.2007.03.015

Buckingham M, Vincent SD (2009) Distinct and dynamic myogenic populations in the vertebrate embryo. Curr Opin Genet Dev 19:444–453. doi:10.1016/j.gde.2009.08.001

Buckingham M, Meilhac S, Zaffran S (2005) Building the mammalian heart from two sources of myocardial cells. Nat Rev Genet 6:826–835. doi:10.1038/nrg1710

Burke AC, Nelson CE, Morgan BA, Tabin C (1995) Hox genes and the evolution of vertebrate axial morphology. Development 121:333–346

Cai CL, Liang X, Shi Y, Chu PH, Pfaff SL, Chen J, Evans S (2003) Isl1 identifies a cardiac progenitor population that proliferates prior to differentiation and contributes a majority of cells to the heart. Dev Cell 5:877–889

Chankiewitz V, Morosan-Puopolo G, Yusuf F, Rudloff S, Pröls F, Kleff V, Hofmann DK, Brand-Saberi B (2014) A thymosin beta15-like peptide promotes intersegmental myotome extension in the chicken embryo. Histochem Cell Biol 141:275–87. doi:10.1007/s00418-013-1156-z

Charite J, McFadden DG, Olson EN (2000) The bHLH transcription factor dHAND controls Sonic hedgehog expression and establishment of the zone of polarizing activity during limb development. Development 127:2461–2470

Chevallier A, Kieny M, Mauger A (1977) Limb-somite relationship: origin of the limb musculature. J Embryol Exp Morphol 41:245–258

Christ B, Jacob HJ, Jacob M (1977) [Experimental findings on muscle development in the limbs of the chick embryo]. Verh Anat Ges 1231–1237

Christ B, Ordahl CP (1995) Early stages of chick somite development. Anat Embryol (Berl) 191:381–396

Collins CA, Olsen I, Zammit PS, Heslop L, Petrie A, Partridge TA, Morgan JE (2005) Stem cell function, self-renewal, and behavioral heterogeneity of cells from the adult muscle satellite cell niche. Cell 122:289–301. doi:10.1016/j.cell.2005.05.010

Couly GF, Coltey PM, Le Douarin NM (1992) The developmental fate of the cephalic mesoderm in quail-chick chimeras. Development 114:1–15

Couly GF, Coltey PM, Le Douarin NM (1993) The triple origin of skull in higher vertebrates: a study in quail-chick chimeras. Development 117:409–429

Dastjerdi A, Robson L, Walker R et al (2007) Tbx1 regulation of myogenic differentiation in the limb and cranial mesoderm. Dev Dyn 236:353–363. doi:10.1002/dvdy.21010

Deimling SJ, Drysdale TA (2009) Retinoic acid regulates anterior-posterior patterning within the lateral plate mesoderm of Xenopus. Mech Dev 126:913–923. doi:10.1016/j.mod.2009.07.001

Dodou E, Verzi MP, Anderson JP, Xu SM, Black BL (2004) Mef2c is a direct transcriptional target of ISL1 and GATA factors in the anterior heart field during mouse embryonic development. Development 131:3931–3942. doi:10.1242/dev.01256

Dong F, Sun X, Liu W et al (2006) Pitx2 promotes development of splanchnic mesoderm-derived branchiomeric muscle. Development 133:4891–4899. doi:10.1242/dev.02693

Engleka KA, Gitler AD, Zhang M, Zhou DD, High FA, Epstein JA (2005) Insertion of Cre into the Pax3 locus creates a new allele of Splotch and identifies unexpected Pax3 derivatives. Dev Biol 280:396–406. doi:10.1016/j.ydbio.2005.02.002

Ericsson R, Olsson L (2004) Patterns of spatial and temporal visceral arch muscle development in the Mexican axolotl (Ambystoma mexicanum). J Morphol 261:131–140. doi:10.1002/jmor.10151

Ericsson R, Cerny R, Falck P, Olsson L (2004) Role of cranial neural crest cells in visceral arch muscle positioning and morphogenesis in the Mexican axolotl, Ambystoma mexicanum. Dev Dyn 231:237–247. doi:10.1002/dvdy.20127

Ericsson R, Knight R, Johanson Z (2013) Evolution and development of the vertebrate neck. J Anat 222:67–78. doi:10.1111/j.1469-7580.2012.01530.x

Evans DJ, Noden DM (2006) Spatial relations between avian craniofacial neural crest and paraxial mesoderm cells. Dev Dyn 235:1310–1325. doi:10.1002/dvdy.20663

Funayama N, Sato Y, Matsumoto K, Ogura T, Takahashi Y (1999) Coelom formation: binary decision of the lateral plate mesoderm is controlled by the ectoderm. Development 126:4129–4138

Gage PJ, Suh H, Camper SA (1999) The bicoid-related Pitx gene family in development. Mamm Genome 10:197–200

Garg V, Yamagishi C, Hu T, Kathiriya IS, Yamagishi H, Srivastava D (2001) Tbx1, a DiGeorge syndrome candidate gene, is regulated by sonic hedgehog during pharyngeal arch development. Dev Biol 235:62–73. doi:10.1006/dbio.2001.0283

Goulding M, Paquette A (1994) Pax genes and neural tube defects in the mouse. Ciba Found Symp 181:103–113, discussion 113-107

Goulding MD, Chalepakis G, Deutsch U, Erselius JR, Gruss P (1991) Pax-3, a novel murine DNA binding protein expressed during early neurogenesis. EMBO J 10:1135–1147

Goulding M, Sterrer S, Fleming J et al (1993) Analysis of the Pax-3 gene in the mouse mutant splotch. Genomics 17:355–363. doi:10.1006/geno.1993.1332

Gros J, Manceau M, Thome V, Marcelle C (2005) A common somitic origin for embryonic muscle progenitors and satellite cells. Nature 435:954–958. doi:10.1038/nature03572

Halevy O, Piestun Y, Allouh MZ et al (2004) Pattern of Pax7 expression during myogenesis in the posthatch chicken establishes a model for satellite cell differentiation and renewal. Dev Dyn 231:489–502. doi:10.1002/dvdy.20151

Harel I, Nathan E, Tirosh-Finkel L, Zigdon H, Guimaraes-Camboa N, Evans SM, Tzahor E (2009) Distinct origins and genetic programs of head muscle satellite cells. Dev Cell 16:822–832. doi:10.1016/j.devcel.2009.05.007

Huang R, Zhi Q, Ordahl CP, Christ B (1997) The fate of the first avian somite. Anat Embryol (Berl) 195:435–449

Huang R, Zhi Q, Izpisua-Belmonte JC, Christ B, Patel K (1999) Origin and development of the avian tongue muscles. Anat Embryol (Berl) 200:137–152

Huang R, Zhi Q, Patel K, Wilting J, Christ B (2000) Contribution of single somites to the skeleton and muscles of the occipital and cervical regions in avian embryos. Anat Embryol (Berl) 202:375–383

Kelly RG, Jerome-Majewska LA, Papaioannou VE (2004) The del22q11.2 candidate gene Tbx1 regulates branchiomeric myogenesis. Hum Mol Genet 13:2829–2840. doi:10.1093/hmg/ddh304

Kitamura K, Miura H, Miyagawa-Tomita S et al (1999) Mouse Pitx2 deficiency leads to anomalies of the ventral body wall, heart, extra- and periocular mesoderm and right pulmonary isomerism. Development 126:5749–5758

Kontges G, Lumsden A (1996) Rhombencephalic neural crest segmentation is preserved throughout craniofacial ontogeny. Development 122:3229–3242

Kraus F, Haenig B, Kispert A (2001) Cloning and expression analysis of the mouse T-box gene tbx20. Mech Dev 100:87–91

Kuang S, Kuroda K, Le Grand F, Rudnicki MA (2007) Asymmetric self-renewal and commitment of satellite stem cells in muscle. Cell 129:999–1010. doi:10.1016/j.cell.2007.03.044

Laugwitz KL, Moretti A, Lam J et al (2005) Postnatal isl1+ cardioblasts enter fully differentiated cardiomyocyte lineages. Nature 433:647–653. doi:10.1038/nature03215

Lepper C, Conway SJ, Fan CM (2009) Adult satellite cells and embryonic muscle progenitors have distinct genetic requirements. Nature 460:627–631. doi:10.1038/nature08209

Lescroart F, Kelly RG, Le Garrec JF, Nicolas JF, Meilhac SM, Buckingham M (2010) Clonal analysis reveals common lineage relationships between head muscles and second heart field derivatives in the mouse embryo. Development 137:3269–3279. doi:10.1242/dev.050674

Lin Q, Schwarz J, Bucana C, Olson EN (1997) Control of mouse cardiac morphogenesis and myogenesis by transcription factor MEF2C. Science 276:1404–1407

Lu JR, Bassel-Duby R, Hawkins A et al (2002) Control of facial muscle development by MyoR and capsulin. Science 298:2378–2381. doi:10.1126/science.1078273

Mahlapuu M, Ormestad M, Enerback S, Carlsson P (2001) The forkhead transcription factor Foxf1 is required for differentiation of extra-embryonic and lateral plate mesoderm. Development 128:155–166

Marcucio RS, Noden DM (1999) Myotube heterogeneity in developing chick craniofacial skeletal muscles. Dev Dyn 214:178–194. doi:10.1002/(SICI)1097-0177(199903)214:3<178::AID-AJA2>3.0.CO;2-4

Matsuoka T, Ahlberg PE, Kessaris N et al (2005) Neural crest origins of the neck and shoulder. Nature 436:347–355. doi:10.1038/nature03837

Mauro A (1961) Satellite cell of skeletal muscle fibers. J Biophys Biochem Cytol 9:493–495

McGonnell IM (2001) The evolution of the pectoral girdle. J Anat 199:189–194

Montarras D, Morgan J, Collins C et al (2005) Direct isolation of satellite cells for skeletal muscle regeneration. Science 309:2064–2067. doi:10.1126/science.1114758

Moretti A, Caron L, Nakano A et al (2006) Multipotent embryonic isl1+ progenitor cells lead to cardiac, smooth muscle, and endothelial cell diversification. Cell 127:1151–1165. doi:10.1016/j.cell.2006.10.029

Nathan E, Monovich A, Tirosh-Finkel L et al (2008) The contribution of Islet1-expressing splanchnic mesoderm cells to distinct branchiomeric muscles reveals significant heterogeneity in head muscle development. Development 135:647–657. doi:10.1242/dev.007989

Noden DM (1983a) The embryonic origins of avian cephalic and cervical muscles and associated connective tissues. Am J Anat 168:257–276. doi:10.1002/aja.1001680302

Noden DM (1983b) The role of the neural crest in patterning of avian cranial skeletal, connective, and muscle tissues. Dev Biol 96:144–165

Noden DM (1986a) Origins and patterning of craniofacial mesenchymal tissues. J Craniofac Genet Dev Biol Suppl 2:15–31

Noden DM (1986b) Patterning of avian craniofacial muscles. Dev Biol 116:347–356

Noden DM, Francis-West P (2006) The differentiation and morphogenesis of craniofacial muscles. Dev Dyn 235:1194–1218. doi:10.1002/dvdy.20697

Noden DM, Trainor PA (2005) Relations and interactions between cranial mesoderm and neural crest populations. J Anat 207:575–601. doi:10.1111/j.1469-7580.2005.00473.x

Noden DM, Marcucio R, Borycki AG, Emerson CP Jr (1999) Differentiation of avian craniofacial muscles: I. Patterns of early regulatory gene expression and myosin heavy chain synthesis. Dev Dyn 216:96–112. doi:10.1002/(SICI)1097-0177(199910)216:2<96::AID-DVDY2>3.0.CO;2-6

Olsson L, Falck P, Lopez K, Cobb J, Hanken J (2001) Cranial neural crest cells contribute to connective tissue in cranial muscles in the anuran amphibian, Bombina orientalis. Dev Biol 237:354–367. doi:10.1006/dbio.2001.0377

Ono Y, Boldrin L, Knopp P, Morgan JE, Zammit PS (2010) Muscle satellite cells are a functionally heterogeneous population in both somite-derived and branchiomeric muscles. Dev Biol 337:29–41. doi:10.1016/j.ydbio.2009.10.005

Piekarski N, Olsson L (2007) Muscular derivatives of the cranialmost somites revealed by long-term fate mapping in the Mexican axolotl (Ambystoma mexicanum). Evol Dev 9:566–578. doi:10.1111/j.1525-142X.2007.00197.x

Psychoyos D, Stern CD (1996) Fates and migratory routes of primitive streak cells in the chick embryo. Development 122:1523–1534

Relaix F, Rocancourt D, Mansouri A, Buckingham M (2005) A Pax3/Pax7-dependent population of skeletal muscle progenitor cells. Nature 435:948–953. doi:10.1038/nature03594

Reshef R, Maroto M, Lassar AB (1998) Regulation of dorsal somitic cell fates: BMPs and Noggin control the timing and pattern of myogenic regulator expression. Genes Dev 12:290–303

Rinon A, Lazar S, Marshall H et al (2007) Cranial neural crest cells regulate head muscle patterning and differentiation during vertebrate embryogenesis. Development 134:3065–3075. doi:10.1242/dev.002501

Sacco A, Doyonnas R, Kraft P, Vitorovic S, Blau HM (2008) Self-renewal and expansion of single transplanted muscle stem cells. Nature 456:502–506. doi:10.1038/nature07384

Saldivar JR, Sechrist JW, Krull CE, Ruffins S, Bronner-Fraser M (1997) Dorsal hindbrain ablation results in rerouting of neural crest migration and changes in gene expression, but normal hyoid development. Development 124:2729–2739

Sambasivan R, Tajbakhsh S (2007) Skeletal muscle stem cell birth and properties. Semin Cell Dev Biol 18:870–882. doi:10.1016/j.semcdb.2007.09.013

Sambasivan R, Gayraud-Morel B, Dumas G, Cimper C, Paisant S, Kelly RG, Tajbakhsh S (2009) Distinct regulatory cascades govern extraocular and pharyngeal arch muscle progenitor cell fates. Dev Cell 16:810–821. doi:10.1016/j.devcel.2009.05.008

Sambasivan R, Kuratani S, Tajbakhsh S (2011) An eye on the head: the development and evolution of craniofacial muscles. Development 138:2401–2415. doi:10.1242/dev.040972

Scherson T, Serbedzija G, Fraser S, Bronner-Fraser M (1993) Regulative capacity of the cranial neural tube to form neural crest. Development 118:1049–1062

Schienda J, Engleka KA, Jun S et al (2006) Somitic origin of limb muscle satellite and side population cells. Proc Natl Acad Sci U S A 103:945–950. doi:10.1073/pnas.0510164103

Schmidt J, Piekarski N, Olsson L (2013) Cranial muscles in amphibians: development, novelties and the role of cranial neural crest cells. J Anat 222:134–146. doi:10.1111/j.1469-7580.2012.01541.x

Schoenwolf GC, Alvarez IS (1992) Role of cell rearrangement in axial morphogenesis. Curr Top Dev Biol 27:129–173

Schoenwolf GC, Garcia-Martinez V, Dias MS (1992) Mesoderm movement and fate during avian gastrulation and neurulation. Dev Dyn 193:235–248. doi:10.1002/aja.1001930304

Seale P, Sabourin LA, Girgis-Gabardo A, Mansouri A, Gruss P, Rudnicki MA (2000) Pax7 is required for the specification of myogenic satellite cells. Cell 102:777–786

Selleck MA, Stern CD (1991) Fate mapping and cell lineage analysis of Hensen's node in the chick embryo. Development 112:615–626

Shih HP, Gross MK, Kioussi C (2007a) Cranial muscle defects of Pitx2 mutants result from specification defects in the first branchial arch. Proc Natl Acad Sci U S A 104:5907–5912. doi:10.1073/pnas.0701122104

Shih HP, Gross MK, Kioussi C (2007b) Expression pattern of the homeodomain transcription factor Pitx2 during muscle development. Gene Expr Patterns 7:441–451. doi:10.1016/j.modgep.2006.11.004

Soriano P (1999) Generalized lacZ expression with the ROSA26 Cre reporter strain. Nat Genet 21:70–71. doi:10.1038/5007

Srivastava D, Cserjesi P, Olson EN (1995) A subclass of bHLH proteins required for cardiac morphogenesis. Science 270:1995–1999

Stephens TD, Beier RL, Bringhurst DC, Hiatt SR, Prestridge M, Pugmire DE, Willis HJ (1989) Limbness in the early chick embryo lateral plate. Dev Biol 133:1–7

Stockdale FE, Nikovits W Jr, Christ B (2000) Molecular and cellular biology of avian somite development. Dev Dyn 219:304–321. doi:10.1002/1097-0177(2000)9999:9999<::AID-DVDY1057>3.0.CO;2-5

Tajbakhsh S, Rocancourt D, Cossu G, Buckingham M (1997) Redefining the genetic hierarchies controlling skeletal myogenesis: Pax-3 and Myf-5 act upstream of MyoD. Cell 89:127–138

Theis S, Patel K, Valasek P et al (2010) The occipital lateral plate mesoderm is a novel source for vertebrate neck musculature. Development 137:2961–2971. doi:10.1242/dev.049726

Tirosh-Finkel L, Elhanany H, Rinon A, Tzahor E (2006) Mesoderm progenitor cells of common origin contribute to the head musculature and the cardiac outflow tract. Development 133:1943–1953. doi:10.1242/dev.02365

Torrey TW (1978) Morphogenesis of the Vertebrates. Wiley, New York

Tucker AS, Lumsden A (2004) Neural crest cells provide species-specific patterning information in the developing branchial skeleton. Evol Dev 6:32–40

Tzahor E (2009) Heart and craniofacial muscle development: a new developmental theme of distinct myogenic fields. Dev Biol 327:273–279. doi:10.1016/j.ydbio.2008.12.035

Tzahor E, Kempf H, Mootoosamy RC et al (2003) Antagonists of Wnt and BMP signaling promote the formation of vertebrate head muscle. Genes Dev 17:3087–3099. doi:10.1101/gad.1154103

Vaglia JL, Hall BK (1999) Regulation of neural crest cell populations: occurrence, distribution and underlying mechanisms. Int J Dev Biol 43:95–110

Valasek P, Evans DJ, Maina F, Grim M, Patel K (2005) A dual fate of the hindlimb muscle mass: cloacal/perineal musculature develops from leg muscle cells. Development 132:447–458. doi:10.1242/dev.01545

Valasek P, Theis S, Krejci E et al (2010) Somitic origin of the medial border of the mammalian scapula and its homology to the avian scapula blade. J Anat 216:482–488. doi:10.1111/j.1469-7580.2009.01200.x

Verzi MP, McCulley DJ, De Val S, Dodou E, Black BL (2005) The right ventricle, outflow tract, and ventricular septum comprise a restricted expression domain within the secondary/anterior heart field. Dev Biol 287:134–145. doi:10.1016/j.ydbio.2005.08.041

Wachtler F, Jacob M (1986) Origin and development of the cranial skeletal muscles. Bibl Anat 24–46

Wachtler F, Jacob HJ, Jacob M, Christ B (1984) The extrinsic ocular muscles in birds are derived from the prechordal plate. Naturwissenschaften 71:379–380

Waxman JS, Keegan BR, Roberts RW, Poss KD, Yelon D (2008) Hoxb5b acts downstream of retinoic acid signaling in the forelimb field to restrict heart field potential in zebrafish. Dev Cell 15:923–934. doi:10.1016/j.devcel.2008.09.009

Yamagishi T, Nakajima Y, Nishimatsu S, Nohno T, Ando K, Nakamura H (2004) Expression of tbx20 RNA during chick heart development. Dev Dyn 230:576–580. doi:10.1002/dvdy.20076

Yelon D, Ticho B, Halpern ME, Ruvinsky I, Ho RK, Silver LM, Stainier DY (2000) The bHLH transcription factor hand2 plays parallel roles in zebrafish heart and pectoral fin development. Development 127:2573–2582

Young JZ (1981) The life of vertebrates, 3rd edn. Clarendon, Oxford

Zammit PS, Golding JP, Nagata Y, Hudon V, Partridge TA, Beauchamp JR (2004) Muscle satellite cells adopt divergent fates: a mechanism for self-renewal? J Cell Biol 166:347–357. doi:10.1083/jcb.200312007

Zammit PS, Partridge TA, Yablonka-Reuveni Z (2006) The skeletal muscle satellite cell: the stem cell that came in from the cold. J Histochem Cytochem 54:1177–1191. doi:10.1369/jhc.6R6995.2006

Zhao X, Sirbu IO, Mic FA, Molotkova N, Molotkov A, Kumar S, Duester G (2009) Retinoic acid promotes limb induction through effects on body axis extension but is unnecessary for limb patterning. Curr Biol 19:1050–1057. doi:10.1016/j.cub.2009.04.059

Regulation of Skeletal Muscle Development and Disease by microRNAs

Ning Liu and Rhonda Bassel-Duby

Abstract The identification of microRNAs (miRNA) in vertebrates has uncovered new mechanisms regulating skeletal muscle development and disease. miRNAs are inhibitors and act by silencing specific mRNAs or by repressing protein translation. In many cases, miRNAs are involved in physiological or pathological stress, suggesting they function to exacerbate or protect the organism during stress or disease. Although many skeletal muscle diseases differ in clinical and pathological manifestations, they all have a common feature of dysregulation of miRNA expression. In particular, analysis of miRNA expression patterns in skeletal muscle diseases reveals miRNA signatures, showing many miRNAs are dysregulated during disease. Emerging identification of miRNA targets and involvement in genetic regulatory networks serve to reveal new regulatory pathways in skeletal muscle biology. This chapter features the findings pertaining to skeletal muscle miRNAs in skeletal muscle development and disease and highlights therapeutic applications of miRNA-based technology in diagnosis and treatment of skeletal muscle myopathies.

1 miRNA Biogenesis and Mechanisms of Action

miRNAs are a class of ~22 nucleotide, small noncoding RNAs that are evolutionarily conserved from plants to mammals (Bartel 2004). The human genome is estimated to encode as many as 1,000 miRNAs, which are either transcribed from their own transcriptional units or embedded in the introns of protein-coding genes and cotranscribed with host genes (Bartel 2004).

miRNAs are transcribed by RNA polymerase II as pri-miRNAs encoding one or multiple miRNAs (Lee et al. 2003; Cai et al. 2004). Pri-miRNAs are processed in the nucleus by the endonuclease Drosha and its cofactor DGCR8 into

N. Liu (✉) • R. Bassel-Duby
Department of Molecular Biology, University of Texas Southwestern Medical Center, Dallas, TX 75390, USA
e-mail: ning.liu@utsouthwestern.edu; rhonda.bassel-duby@utsouthwestern.edu

© Springer-Verlag Berlin Heidelberg 2015

B. Brand-Saberi (ed.), *Vertebrate Myogenesis*, Results and Problems in Cell Differentiation 56, DOI 10.1007/978-3-662-44608-9_8

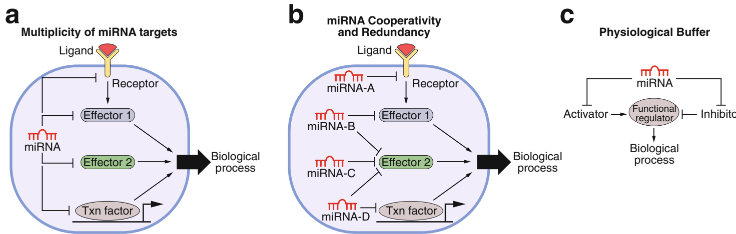


Fig. 1 Mechanisms of action of miRNAs. (**a**) Multiplicity of mRNA targets. (**b**) miRNA cooperativity and redundancy. (**c**) Physiological buffer

~70 nucleotide hairpins, known as pre-miRNA stem loops (Lee et al. 2003; Denli et al. 2004; Gregory et al. 2004). The pre-miRNAs are exported to the nuclease where they are processed by the endonuclease Dicer to yield imperfect RNA duplexes containing miRNAs (Chendrimada et al. 2005). The mature miRNA is released from Dicer and incorporated into the RNA-induced silencing complex (RISC) where it binds to the 3′ untranslated regions (UTR) of target mRNAs via imperfect Watson–Crick base-pairing, repressing its expression by translational inhibition and mRNA degradation (Filipowicz et al. 2008).

miRNA-dependent gene regulation is a complex and highly orchestrated process. Most of the miRNAs exert their inhibitory effects through subtle modulations of their targets, referred to as "fine-tuning," instead of acting as an "on-and-off" switch (Bartel 2009). A single miRNA can repress several mRNAs in a common biological pathway, which reduces the dependence on a single miRNA–mRNA interaction and increases the robustness of controlling a gene regulatory network (Fig. 1) (Small and Olson 2011). Many miRNAs may cooperatively or redundantly regulate a single biological process, by individually targeting many components of that process or by synergistically repressing a crucial component of a pathway (Fig. 1) (Small and Olson 2011). In addition, miRNAs may act as a "buffer" against minor perturbations in a biological pathway. This is accomplished by the targeting of factors that positively and negatively influence a particular process, thereby insulating that process from environmental fluctuations (Fig. 1) (Small and Olson 2011).

Studies in *C. elegans* revealed significant redundancy within miRNA families, between unrelated miRNAs, and even between miRNAs and transcription factors (Ambros 2010; Brenner et al. 2010). This extent of redundancy may explain the fact that very few developmental processes are absolutely dependent on a single miRNA (Alvarez-Saavedra and Horvitz 2010; Liu and Olson 2010). Intriguingly, expression and actions of miRNAs are often sensitized under pathological and physiological stress, implicating a more pronounced role of miRNAs in exacerbating or protecting the organism during stress or disease (Small and Olson 2011).

2 miRNA in Muscle Development and Function

The requirement of miRNAs for skeletal muscle development and function was initially demonstrated in mice by a tissue-specific deletion of the Dicer gene, which encodes an enzyme essential for miRNA biogenesis. Deletion of a conditional Dicer allele in embryonic skeletal muscle using a MyoD Cre recombinase transgene results in skeletal muscle hypoplasia (a decrease in the number of myofibers), increased apoptosis, and lethality within minutes following birth (O'Rourke et al. 2007). In this study, expression of many muscle-specific miRNAs is downregulated in skeletal muscle (O'Rourke et al. 2007). The severe phenotype in mice lacking Dicer in skeletal muscle is likely due to the absence of the collective functions of numerous miRNAs rather than the action of a single miRNA in skeletal muscle development.

2.1 miRNA in Muscle Development

Skeletal muscle-enriched miRNAs are referred to as MyomiRs and include miR-1, miR-133a, miR-133b, miR-206, miR-208b, and miR-499 (McCarthy and Esser 2007; van Rooij et al. 2009). The miR-1/206 family is comprised of miR-1-1, miR-1-2, and miR-206. The miR-133 family is comprised of miR-133a-1, miR-133a-2, and miR-133b (Liu and Olson 2010). These miRNAs are cotranscribed from bicistronic transcripts on three separate chromosomes. miR-1-1 and miR-1-2 are identical and differ from miR-206 by four nucleotides, while miR-133a-1 and miR-133a-2 are identical and differ from miR-133b by two nucleotides. Skeletal muscle expression of miR-1-1/133a-2 and miR-1-2/133a-1 is controlled by combinations of transcription factors, including serum response factor (SRF), MyoD, and myocyte enhancer factor-2 (MEF2) (Zhao et al. 2005; Chen et al. 2006; Liu et al. 2007). MyoD also directly activates transcription of miR-206/133b in skeletal muscle by binding to the E-box in the upstream regulatory region (Rao et al. 2006). Thus, the same transcription factors that activate protein-coding genes involved in muscle function, such as the sarcomeric genes, also regulate muscle-specific miRNAs, demonstrating an interconnected relationship between muscle-specific miRNAs and muscle-specific mRNAs.

The miRNAs in the miR-1/206 family play key roles in myoblast differentiation and muscle development. When myoblasts differentiate into myotubes in cell culture, expression of miR-1 and miR-133 is upregulated. miR-1 promotes myoblast differentiation by targeting and repressing histone deacetylase 4 (HDAC4), a repressor of the transcription factor, MEF2 (Chen et al. 2006). Thus, the interaction between miR-1 and HDAC4 provides a positive feed-forward loop in which MEF2 upregulates the expression of miR-1 causing repression of HDAC4 and ultimately increasing activity of MEF2, which drives myocyte differentiation. In *Drosophila*, miR-1 is required for postmitotic growth of larval muscle. Moreover, the loss of miR-1 in *Drosophila* results in a severely deformed musculature (Kwon et al. 2005;

Sokol and Ambros 2005). In zebrafish, downregulation of both miR-1 and miR-133 alters muscle gene expression and disrupts actin organization during sarcomere assembly, suggesting that miR-1 and miR-133 actively shape gene expression patterns in skeletal muscle by regulating sarcomeric actin organization (Mishima et al. 2009).

miR-206 is also upregulated during myoblast differentiation. It is believed to induce differentiation by repressing a subunit of DNA polymerase alpha (Pola1), connexin 43, as well as follistatin-like 1 (Fstl1) and utrophin (Anderson et al. 2006; Kim et al. 2006; Rosenberg et al. 2006; Hirai et al. 2010). However, the in vivo significance of miR-206 in regulating myoblast differentiation remains a conundrum given that mice lacking miR-206 have normal skeletal muscle development (Williams et al. 2009).

Similar to miR-1 and miR-206, miR-133a expression is also upregulated upon C_2C_{12} myoblast differentiation (Chen et al. 2006). However, in contrast to these two miRNAs, miR-133a promotes myoblast proliferation, at least partly, by repressing SRF (Chen et al. 2006). The genetic interaction between miR-133a and SRF constitutes a negative feedback loop in which the upregulation of miR-133a by SRF results in increased repression of SRF.

2.2 miRNA in Muscle Homeostasis and Function

Skeletal muscle is comprised of heterogeneous myofibers that differ in their physiological and metabolic parameters, enabling different muscle groups to provide a variety of functional properties. The myofibers (types I, IIa, IIb, and IId/x) are classified based on the specific myosin heavy-chain isoform expression. Interestingly, various MyomiRs (miR-208, miR-208b, and miR-499) are embedded in the introns of three muscle-specific myosin heavy-chain genes (Myh6, Myh7, and Myh7b) (van Rooij et al. 2009). These three miRNAs share significant homology in seed sequences, implying that they may have overlapping functions by their regulation of the same set of targets. In skeletal muscle, miR-208b and miR-499 redundantly control muscle fiber identity by activating type-I and repressing type-II myofiber genes. Mice lacking both miR-208b and miR-499 showed a substantial loss of type-I myofibers in the soleus muscle (van Rooij et al. 2009). Conversely, forced expression of miR-499 in skeletal muscle induces a complete conversion of all type-II myofibers in soleus to type-I fibers (van Rooij et al. 2009). These skeletal muscle MyomiRs target a collection of transcriptional repressors of type-I muscle genes, including Sox6, Purß, and Sp3. In fact, conditional deletion of Sox6 in neonatal skeletal muscle in mice leads to a myofiber conversion (type II to type I), accompanied by changes in skeletal muscle mechanics and performance (Quiat et al. 2011). These studies demonstrate the important roles of MyomiRs in regulating skeletal muscle gene program and muscle performance.

In a mouse model of skeletal muscle hypertrophy, expression of miR-1 and miR-133a is decreased (McCarthy and Esser 2007). However, it is unclear whether

manipulation of miR-1 expression level has consequential effects on skeletal muscle hypertrophy. Interestingly, a mutation that is responsible for the exceptional muscularity of Texel sheep has been mapped to a single G-to-A mutation in the 3′ UTR of the myostatin gene, which creates a binding site for miR-1 and miR-206 (Clop et al. 2006). Myostatin functions to repress muscle growth, and the translational repression of myostatin by miR-1/206 is believed to contribute to the muscular hypertrophy of Texel sheep. These findings implicate a role for miRNAs in skeletal muscle hypertrophy.

Recent studies have indicated that MyomiRs are involved in metabolic and structure programs controlling muscle fitness and endurance. Using the MCK-PPARβ/δ transgenic mouse line which has a "trained" phenotype, in which both energy metabolic and fiber-type programs linked to muscle endurance are activated (Wang et al. 2004), it was shown that PPARβ/δ functions to activate transcription of the Myh7 and Myh7b genes, increasing the levels of miR-208b and miR-499 and, thereby, triggering a cascade of muscle slow-twitch contractile protein gene expression. This study elegantly identifies a gene regulatory pathway, involving nuclear receptor and miRNA signaling, which is involved in the coordinate control of muscle energy metabolism and fiber type (Gan et al. 2013).

Since exercise puts a mechanical and metabolic stress on skeletal muscle, it is reasonable to expect changes in miRNA expression following exercise. Over the past few years, various studies have documented the regulation of miRNAs by exercise (Kirby and McCarthy 2013; Zacharewicz et al. 2013). For example, expression of miR-23 and miR-696 is decreased in skeletal muscle after endurance training in mice and is increased in the skeletal muscle of immobilized mice (Safdar et al. 2009; Aoi et al. 2010). Both of these miRNAs are shown to negatively regulate metabolism and mitochondrial biogenesis by repressing peroxisome proliferator-activated receptor-γ coactivator-α (PGC-1α), a key metabolic modulator in skeletal muscle. In humans, following 12 weeks of endurance exercise training, expression of myomiRs miR-1, miR-133a, miR-133b, and miR-206 were significantly downregulated and returned to pre-training baseline levels 2 weeks after the cessation of training. With resistance training, it was observed in humans that miR-1 expression is reduced 3 and 6 h following a single bout of exercise, while no changes were observed in miR-133a and miR-206 levels (Drummond et al. 2008). At this point, miRNA expression data are being collected following various exercise programs. To understand the physiological significance of these changing miRNA expression levels in response to exercise, further identification of the miRNA targets in exercise is needed.

2.3 miRNAs in Adult Skeletal Muscle Regeneration

Skeletal muscles possess the remarkable ability to regenerate after injury, exercise, or disease such as muscular dystrophies. This regenerative capacity relies on satellite cells, a heterogeneous population of stem cells and committed progenitor

cells that represent 2-5 % of all myofiber nuclei in adult hindlimb muscle. Satellite cells reside in a niche between the basal lamina and the sarcolemma of their associated muscle fibers. Under normal conditions, adult satellite cells are maintained in a mitotically quiescent state, with limited gene expression and protein synthesis (Charge and Rudnicki 2004; Dhawan and Rando 2005). Once activated by intrinsic and extrinsic signals upon injury or disease, satellite cells leave their niche and move outside of the basal lamina to re-enter cell cycle. Activated satellite cells acquire a myogenic fate and express the myogenic regulatory factors (MRFs) MyoD and Myf5 (Tedesco et al. 2010; Brack and Rando 2012; Yin et al. 2013). After multiple rounds of proliferation, myoblasts start to differentiate to form multinucleated myofibers that fuse with each other and with existing myofibers to recreate functional muscle tissue. In addition, the uncommitted satellite "stem" cells, which never expressed myogenic genes, undergo asymmetric cell division to replenish the reservoir of quiescent stem cells (Kuang et al. 2007).

Paired box 7 transcription factor Pax7 is expressed in both quiescent and activated satellite cells and is required for maintenance and self-renewal of quiescent satellite cells (Buckingham 2007). Pax7 is essential for regulating the expansion and differentiation of satellite cells during both neonatal and adult myogenesis. Deletion of *Pax7* in adult satellite cells results in markedly impaired skeletal muscle regeneration (von Maltzahn et al. 2013). Furthermore, depletion of Pax7-expressing satellite cells by diphtheria toxin completely blocked regeneration following acute injury (Lepper et al. 2011; McCarthy et al. 2011; Murphy et al. 2011; Sambasivan et al. 2011). These studies demonstrated the pivotal role of Pax7 in controlling the identity and function of adult satellite cells.

miRNAs play an essential role in the maintenance of satellite-cell quiescence and in the survival of proliferating myogenic progenitors. Satellite cell-specific deletion of Dicer resulted in spontaneous loss of quiescence and activation of cell cycle and proliferation, as well as extensive apoptosis of satellite cell progeny (Cheung et al. 2012). Among the 22 quiescent-specific miRNAs, miR-489 functions as a regulator of satellite-cell quiescence, by suppressing the oncogene Dek, which promotes the transient expansion of myogenic progenitors (Cheung et al. 2012). In quiescent satellite cells, Myf5 mRNA is localized in mRNP granules, where miR-31 represses its translation. On activation, mRNP granules dissociate, and release Myf5 transcripts from miR-31 suppression, leading to rapid translation and accumulation of the Myf5 protein, which labels activated myoblasts and promotes myogenesis (Crist et al. 2012).

Activation of satellite cells promotes MyoD expression, and in addition to activating the skeletal muscle myogenic pathways, MyoD upregulates expression of miRNAs. In particular, miR-206 is significantly upregulated in activated satellite cells (Cacchiarelli et al. 2010; Chen et al. 2010). Of note, miR-206 targets Pax7 3′UTR and directly repress Pax7 expression to restrict the proliferation of satellite cells and facilitate differentiation. Using miR-206 antagomiRs to specifically knockdown miR-206 expression results in enhanced satellite cell proliferation and increased Pax7 expression, which inhibits differentiation (Cacchiarelli et al. 2010; Chen et al. 2010). Therefore, following activation of satellite cells,

MyoD not only activates myogenic genes that promote differentiation of satellite cells into myotubes but also represses satellite cell survival and self-renewal via direct upregulation of miR-206 expression, thus pushing them toward the differentiation pathway.

In response to cardiotoxin injury of skeletal muscle, satellite cells are activated to initiate the regenerative response. Following cardiotoxin injection into skeletal muscle, microarray analysis identified a number of miRNAs that were dysregulated. miR-206 was the most dramatically upregulated miRNA on day 7 after cardiotoxin delivery into the TA muscle (Liu et al. 2012). miR-206 continued to be strongly expressed throughout the course of muscle regeneration . The increase in miR-206 expression resulted in downregulation of various genes including Pax7, Notch3, IGFBP5 (Liu et al. 2012), and Hmgb3 (Maciotta et al. 2012). Using a genetic deletion of miR-206 it was shown that miR-206 promotes skeletal muscle regeneration in response to injury (Liu et al. 2012). Furthermore, miR-206 expression is enriched in regenerating fibers, implying its involvement in the regeneration process.

Many other miRNAs are also involved in skeletal muscle regeneration. For example, miR-26 promotes myoblast differentiation by suppressing transcription factors Smad1 and Sma4, which are well-known inhibitors of differentiate. Inhibition of miR-26 in adult mice caused a delay in regeneration upon muscle injury (Dey et al. 2012).

3 miRNAs in Primary Muscle Disorders

Primary muscle disorders are a diverse group of muscle diseases that involve muscle fiber degeneration and regeneration, inflammation, and muscle weakness. In general, there are three groups of diseases: (1) the muscular dystrophies, which include Duchenne muscular dystrophy (DMD), Becker muscular dystrophy (BMD), Limb-Girdle muscular dystrophies (LGMD), Myotonic dystrophy (DM), Facioscapulohumeral muscular dystrophy (FSHD), congenital muscular dystrophies (CMDs), and Emery-Dreifuss muscular dystrophy (EDMD); (2) congenital myopathies, including nemalin myopathy and centronuclear myopathies; and (3) inflammatory myopathies, including polymyositis, dermatomyositis, and inclusion-body myositism, all of which involve inflammation of the muscle. Although their clinical and pathological manifestations are different, these muscle diseases are all caused by various mutations in the myofibers, which include structural proteins, signaling molecules, enzymes, and proteins involved in posttranscriptional regulation.

Another striking but common feature of these primary muscle diseases is dysregulation of miRNA expression. Gene profiling analysis revealed that 185 miRNAs are differentially expressed in at least 1 or 10 major muscular disorders in humans (Eisenberg et al. 2007). Among them, 5 miRNAs (miR-146b, miR-221, miR-155, miR-214, and miR-222) are consistently

dysregulated in all ten diseases, suggesting a common regulatory mechanism in these diseases (Eisenberg et al. 2007). The majority of these miRNAs are differentially expressed in only one disorder, pointing to the specific regulation of individual miRNA in specific pathological disease pathway. In this section, we will highlight the involvement of miRNAs in these muscle disorders.

3.1 miRNAs in the Muscular Dystrophies

3.1.1 miRNAs in DMD Patients and *mdx* Mice

Duchenne muscular dystrophy (DMD), the most common and severe form of muscular dystrophy, is a recessive X-linked inherited disorder, affecting 1 in 3,500 live male births (Blake et al. 2002). Most affected boys are diagnosed in the first few years of life and display delayed walking, falling, and a toe gait and calf hypertrophy. DMD is caused by mutations in the dystrophin gene on the X-chromosome, the largest gene in humans (Hoffman et al. 1987; Chamberlain et al. 1988). Dystrophin is the core component of the dystrophin-associated glycoprotein complex (DAPC), which links the intracellular cytoskeleton of the myofibers to the extracellular matrix structural proteins (Davies and Nowak 2006). Loss of dystrophin expression in DMD patients causes fragility of myofibers to mechanical damage, leading to activation of satellite cells (myogenic stem cells) and myofiber regeneration (Wallace and McNally 2009). However, the unsustainable activation of satellite cells in DMD patients ultimately results in severe muscle wasting, infiltration of adipocytes, inflammation, and eventual paralysis and death (McNally and Pytel 2007). The milder phenotype of Becker muscular dystrophy (BMD) is also caused by mutations in dystrophin, although BMD patients often have partially functional dystrophin protein (Wallace and McNally 2009).

Mdx mice, which harbor a premature termination codon in the dystrophin gene, are the most commonly used mouse model of DMD (Chamberlain and Banks 2008). The *mdx* mouse has provided important insights into the pathological mechanisms of DMD. Intriguingly, despite sharing the same genetic defects as DMD patients, *mdx* mice display a relatively mild and slowly progressive dystrophic phenotype with normal life span, which has limited the usefulness of this model for therapeutic development for DMD patients. Interestingly, secondary gene mutations in *mdx* mice, such as mutations in utrophin, α-dystrobrevin, α7-integrin, result in more severe dystrophic phenotypes, highlighting the importance of other cellular components in DMD disease progression (Deconinck et al. 1997; Grady et al. 1997; Grady et al. 1999; Guo et al. 2006; Chandrasekharan et al. 2010).

Comparison of miRNA expression patterns in DMD patients and *mdx* mice revealed a common miRNA signature, with nearly a dozen miRNAs dysregulated in both disease models (Greco et al. 2009). These dysregulated miRNAs are involved in muscle degeneration, regeneration, and inflammatory responses.

Among the dysregulated miRNAs, miR-206 is of particular interest. miR-206 is a muscle-specific miRNA, strongly upregulated in activated satellite cells during muscle regeneration (Liu et al. 2012). miR-206 promotes muscle regeneration by facilitating activated satellite cells to differentiate into multinucleated myotubes (Liu et al. 2012). Genetic deletion of miR-206 in mice substantially delays regeneration induced by cardiotoxin injury (Liu et al. 2012).

In *mdx* mice, miR-206 is highly upregulated in all muscles at 4 weeks of age, synchronously with the onset of disease (Liu et al. 2012). Satellite cells in *mdx* mice are continuously activated to generate new myofibers to repair damaged and degenerated myofibers caused by loss of expression of the dystrophin gene (Wallace and McNally 2009). Strong activation of miR-206 in the newly formed myofibers in the *mdx* mice made it a potential modifier of the *mdx* phenotype. Indeed, genetic deletion of miR-206 in *mdx* (miR-206-KO; *mdx*) mice resulted in the acceleration and exacerbation of muscle dysfunction. At 4 weeks of age, small regenerating fibers and inflammatory cells were only occasionally observed in *mdx* mice. Strikingly, nearly all the miR-206-KO; *mdx* mice showed extensive myofiber damage and degeneration, with calcium deposition, mineralization and fibrosis (Fig. 2a). Massive accumulation of small regenerating fibers and inflammatory cells were also observed in miR-206-KO; *mdx* mice. The dystrophic phenotype became more apparent at 6 weeks of age, when approximately 17 % of miR-206-KO; *mdx* mice became severely runted, showed kyphosis, and died. In contrast, only 5 % of *mdx* mice died at this age (Fig. 2b).

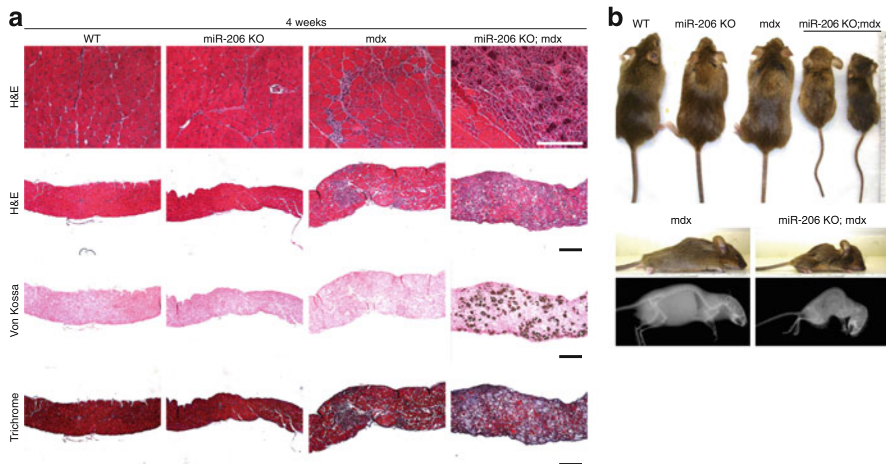


Fig. 2 Loss of miR-206 exacerbates the dystrophic phenotype in mdx mice. (**a**) H&E staining of quadriceps and diaphragm muscle of WT, miR-206 KO, *mdx*, and miR-206 KO; *mdx* mice at 4 weeks of age. *Top two panels*; H&E staining of quadriceps and diaphragm muscles, respectively. *Bottom two panels*: Von Kossa staining and Masson's trichrome staining of diaphragm muscle showing mineralization and fibrosis in diaphragm muscle fibers. Size bar: 200 μm. (**b**) WT, miR-206 KO; *mdx*, and miR-206 KO; *mdx* mice at 6 weeks of age. miR-206 KO; *mdx* mice are runted with kyphosis compared to *mdx* mice. X-ray reveals kyphosis in miR-206 KO; *mdx* mice

These results demonstrate that miR-206 plays a protective role in the setting of muscular dystrophy. The strong activation of miR-206 in *mdx* mice serves as a compensatory mechanism to promote formation of new myofibers in response to muscle damage and injury. In the absence of miR-206, the delayed regeneration and myogenic differentiation result in fibrosis and fatty infiltration as well as mineralization of myofibers, which disrupt muscle integrity and function.

In addition to sustained muscle damage, DMD patients also show extensive fibrosis, replacing muscle with collagenous sclerotic tissues, which aggravates disease severity in patients at advanced stages. Fibrosis is a complex process characterized by excessive accumulation of collagens, elastin, and other extracellular matrix (ECM) components. Fibrosis by dysregulated collagen metabolism in DMD patients leads to the disruption of muscle structure and irreversible loss of normal muscle function. In addition, it represents a major obstacle for the success of the ongoing preclinical therapies at advanced stages of DMD. Therefore, reducing fibrosis is a great challenge in treating DMD patients.

Among numerous miRNAs dysregulated in DMD and *mdx* mice, two miRNAs: miR-29 and miR-21 are of particular interest because of their involvement in fibrosis. The miR-29 family consists of 3 highly homologous miRNAs: miR-29a, b, c, all of which are expressed in various tissues, including skeletal muscle and heart. In the heart, miR-29 acts as a regulator of cardiac fibrosis in response to myocardial infarction by directly repressing collagens and elastin (van Rooij et al. 2008b). Similarly, miR-29 expression is downregulated in muscles of DMD patients and *mdx* mice (Cacchiarelli et al. 2010; Wang et al. 2012). Intriguingly, electroporation of miR-29 expressing plasmids or miR-29 mimic oligonucleotides into hindlimb muscles of *mdx* mice resulted in a significant decrease in collagen deposition, fibrosis, as well as accelerated regeneration of satellite cells. The functional recovery of the *mdx* phenotype following miR-29 injection points to miR-29 as a crucial player in the control of extracellular matrix modification in *mdx* mice. miR-29 replacement therapy might serve as a promising treatment approach for DMD.

miR-21 is specifically expressed in fibroblasts. In *mdx* mice, miR-21 expression is upregulated, and this increased expression is age dependent. miR-21 expression is higher in older than younger *mdx* mice, which correlates with gradual progression of fibrosis in older *mdx* mice (Ardite et al. 2012). Inhibition of miR-21 by antagomiR-21 injection prevented collagen and fibronectin accumulation and fibroblast number, plus, improved muscle homeostasis in aged *mdx* mice (24-months-old) was seen (Ardite et al. 2012). Conversely, miR-21 overexpression by mimic-miR-21 in young *mdx* mice (3 months old) exacerbated fibrosis (Ardite et al. 2012). These studies established the essential role of miR-21 in the fibrotic response in *mdx* mice and indicated that miR-21 silencing can be a potential therapy in treating fibrosis in *mdx* mice.

There are other miRNAs that are dysregulated in DMD patients and *mdx* mice, suggestive of their involvement in disease progression. For example, miR-31 expression is highly regulated in regenerating myofibers in DMD patients and *mdx* mice (Cacchiarelli et al. 2011). miR-31 represses dystrophin expression by

targeting the 3′ UTR of dystrophin mRNA, and inhibition of miR-31 in human DMD myoblasts can increase dystrophin rescue (Cacchiarelli et al. 2011). In addition, miR-486 is downregulated in both *mdx* mice and DMD patients (Alexander et al. 2011). Disruption of miR-486 expression in myoblasts affects myoblast proliferation, migration, and wound healing (Alexander et al. 2011). Overexpression of miR-486 in mice results in impaired muscle regeneration due to its role in cell cycle kinetics. miR-199 is strongly upregulated in DMD patients, *mdx* mice and dystrophin-deficient zebrafish, in a serum response factor (SRF)-dependent manner, along with myocardin-related transcription factors (Alexander et al. 2013). miR-199a regulates myogenic cell proliferation and differentiation in myoblasts. Overexpression of miR-199 in zebrafish resulted in abnormal myofiber disruption and sarcolemmal membrane detachment (Alexander et al. 2013). However, it remains to be determined whether overexpression of miR-486 or inhibition of miR-199 in *mdx* mice will rescue the dystrophic phenotype.

3.1.2 miRNAs in Myotonic Dystrophy Type 1 and 2 (DM1 and DM2)

Myotonic dystrophy type 1 (DM1, or Steinert's disease) is one of the most common autosomal-dominant genetic disorders affecting 1:8,000 individuals (McNally and Pytel 2007). DM1 is characterized by progressive muscle weakness, myotonia, and it also affects other organ functions, including heart, central nervous system, eye, and smooth muscle. DM1 is associated with a trinucleotide (CTG) repeat expansion in the 3′UTR of the DMPK (myotonic dystrophy protein kinase) gene, resulting in production and accumulation of CUG repeat-containing RNA in the nuclei of DM1 patients (McNally and Pytel 2007). DM2 is a milder clinical form of DM1, with a later onset and less severe symptoms. DM2 is caused by a (CCTG)n repeat within the first intron of the zinc finger-9 (ZNF9) gene (Liquori et al. 2001).

miR-1 and miR-335 were upregulated, whereas miR-29b and c, and miR-33 were downregulated in biopsies from 15 DM1 patients compared to control individuals (Perbellini et al. 2011). Interestingly, in addition to expression level change, the cellular distribution of muscle-specific miR-1, miR-133b, and miR-206 was severely altered in DM1 skeletal muscles (Perbellini et al. 2011). Of note, expression levels of predicted targets of miR-1 and miR-29 were also altered, suggesting that dysregulation of miRNAs was likely functionally relevant. In DM2 patients, 11 miRNAs were deregulated: 9 displayed higher levels compared to controls (miR-34a-5p, miR-34b-3p, miR-34c-5p, miR-146b-5p, miR-208a, miR-221-3p, and miR-381), while 4 were decreased (miR-125b-5p, miR-193a-3p, miR-193b-3p, and miR-378a-3p) (Greco et al. 2012). Furthermore, analysis of global gene expression in DM2 highlighted the involvement of the miRNA-deregulated mRNAs in multiple aspects of DM2 pathophysiology, suggesting that miRNA dysregulations may contribute to DM2 pathogenetic mechanisms.

3.2 miRNAs in Congenital Myopathies

Congenital myopathy is a group of rare, inherited, primary muscle disorders that cause hypotonia and weakness at birth or during the neonatal period and, in some cases, delayed motor development later in childhood (Nance et al. 2012). This group includes nemalin myopathy, centronuclear myopathy, and congenital fiber type disproportion myopathy.

Nemaline myopathy (NM), the most common congenital myopathy, is characterized by relatively nonprogressive proximal weakness of congenital onset and the presence of nemaline rod structures in the affected myofibers (Agrawal et al. 2007). Causative mutations have been identified in six genes and all are related to the production of thin-filament proteins, indicating the high genetic heterogeneity of the disease (Agrawal et al. 2007). It is therefore not surprising that NM shows the most extensive dysregulation of miRNAs, compared to other muscle disorders (Eisenberg et al. 2007). More than 150 miRNAs are dysregulated and of these 36 being dysregulated specifically in NM (Eisenberg et al. 2007).

Centronuclear myopathy (CNM) is another group of congenital myopathies characterized by the presence of an abnormally high number of muscle fibers with centrally place nuclei (Jungbluth et al. 2008; Romero 2010). Interestingly, unlike other muscle disorders, signs of necrosis or excessive regeneration are usually absent in all forms of CNM. CNM can be caused by mutations in several genes, such as myotubularin (MTM1) gene (myotubular myopathy), dynamin 2 (DNM2) gene (DNM2-related CNM), and amphiphysin 2 (BIN1-related CNM).

miR-133 family is comprised of three nearly identical miRNAs: miR-133a-1, miR-133a-2, both of which are identical in mature sequence, and miR-133b, which differs from miR-133a-1 by only 2 nucleotides at the 3′ end. miR-133a-1 and miR-133a-1 are specifically expressed in heart and skeletal muscle, whereas miR-133b is exclusively expressed in skeletal muscle. Studies from mice lacking both miR-133a-1 and miR-133a-2 (hence called dKO mice) have revealed their important role in heart development and function. Interestingly, in adult skeletal muscle, dKO mice showed a high proportion of myofibers with centralized nuclei in fast (type II) fibers (Fig. 3) (Liu et al. 2011). In addition, dKO mice were significantly smaller in body mass, muscle mass, as well as myofiber diameters. dKO myofibers showed no sign of muscle damage and degeneration, or inflammation, fibrosis, or apoptosis (Fig. 3) (Liu et al. 2011). In addition, analysis of genes involved in skeletal muscle regeneration indicates that regeneration in dKO muscle is rare, which is insufficient to account for the extensive centronuclear fibers (Liu et al. 2011). dKO skeletal myofibers also showed disorganized triads where excitation–contraction (E–C) coupling occurs, as well as mitochondrial dysfunction and fast-to slow myofiber conversion (Liu et al. 2011).

The pathological phenotype of dKO mice is reminiscent of human DNM2-related CNM. Dynamin 2 encodes a ubiquitously expressed large GTPase that functions in a variety of cellular processes including endocytosis, exocytosis, intracellular membrane trafficking, and actin and myotubule networks (Durieux

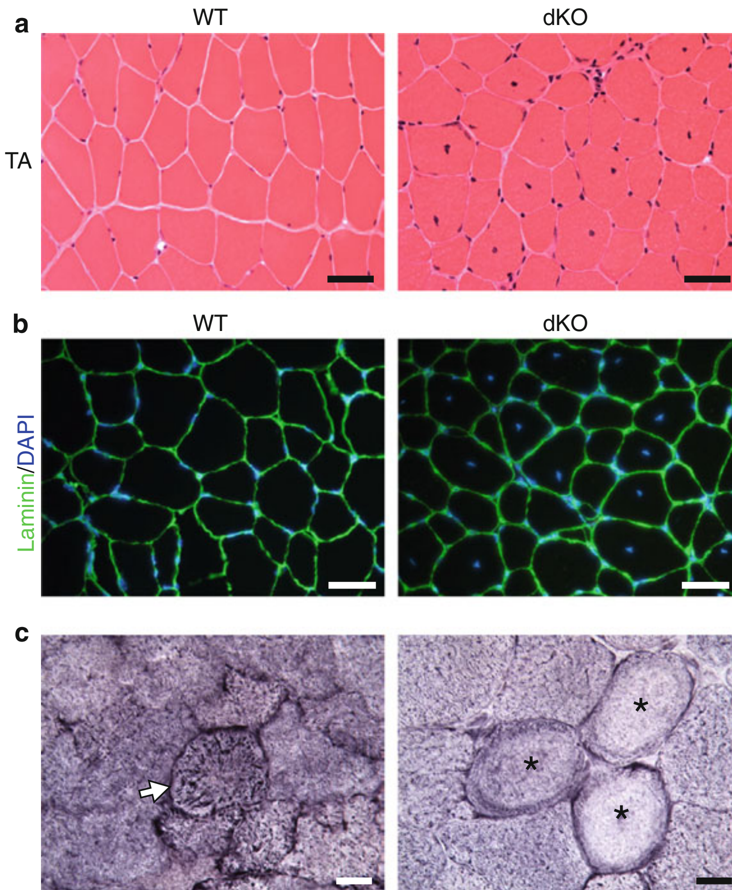


Fig. 3 Centronuclear myopathy in skeletal muscle of miR-133a dKO mice**.** *Top panel*: Hematoxylin and eosin (H&E) stain showing centralized nuclei in TA myofibers of dKO mice. *Middle panel*: Immunostaining of TA muscle against laminin. Nuclei are stained with DAPI. dKO TA muscle shows central nuclei. *Bottom panel*: NADH-TR staining of dKO TA muscle revealed radiating intermyofibrillary network (*arrows*) and ring-like fibers (*asterisks*)

et al. 2010). Mutations in the DNM2 gene have been linked to both Charcot-Marie-Tooth peripheral neuropathy (CMT) and CNM (Durieux et al. 2010). Most of CNM mutations do not affect dynamin 2 mRNA level, protein expression, or localization. In fact, it is now believed these dynamin 2 mutations function in a dominant negative form, or even a superactive form, in some cases (Durieux et al. 2010). Interestingly, miR-133a is shown to target and repress dynamin 2 expression by binding to its 3′ UTR and dynamin 2 expression is upregulated at both mRNA and protein levels in dKO muscle (Liu et al. 2011). Moreover, elevated expression of dynamin 2 in skeletal muscle causes CNM, similar to the dKO muscle (Liu et al. 2011). These results demonstrate that the CNM observed in dKO muscle can be attributed, at least in part, to dysregulation of dynamin 2. Taken together,

these findings highlight the critical role of miR-133a in maintaining normal structure and function of adult skeletal muscle. It remains to be determined whether the level of miR-133 expression in patients with DNM2-related CNM is altered or not.

3.3 miRNAs in Inflammatory Myopathies

Inflammatory myopathies include dermatomyositis (DM), polymyositis (PM), and sporadic inclusion-body myositis (IBM) (Dalakas 2006). The hallmark histopathologic markers common to all these disorders are inflammation of the endomysium (the delicate sheath of reticular fibrils that surrounds each muscle fiber), muscle-fiber necrosis, and fibrosis. DM is a complement-mediated microangiopathy, affecting children and adults with proximal muscle weakness and typical skin changes (Dalakas 2006). PM, the most common form of inflammatory myopathies, is caused by expansion of cytotoxic T cells that surround and invade muscle fibers (Dalakas 2006). In IBM, in addition to T-cell-mediated cytotoxicity, there is vacuolar formation and accumulation of tubulofilamentous inclusions, both of which cause damages of proximal and distal muscles in adults (Dalakas 2006).

Interestingly, the general distinction between inflammatory myopathies and non-immune-mediated muscle disorders becomes less defined, as clinical and histo-pathological overlap between these two groups of disease is being increasingly recognized. Similar to other muscle disorders, a score of miRNAs are upregulated in DM/PM/IBM (Eisenberg et al. 2007). Intriguingly, analysis of predicated target genes of these dysregulated miRNAs revealed a significant over-representation of genes involved in MAPK- and WNT-signaling pathways and immune responses, such as T cell signaling pathway (Eisenberg et al. 2007). This correlation indicates possible involvement of these signaling pathways in the disease progression.

4 miRNAs in Amyotrophic Lateral Sclerosis

Amyotrophic lateral sclerosis (ALS), also known as Lou Gehrig's disease, is a progressive, age-dependent neurodegenerative disease, in which motor neurons within the brain and spinal cord degenerate (Bruijn et al. 2004). Dysfunction and eventual death of motor neurons lead to muscle atrophy, paralysis of lower limb and respiratory muscles and death (Dunckley et al. 2007). While the majority of cases are sporadic, 5–10 % are familiar and are caused by mutations in a variety of genes, such as superoxide dismutase 1 (*SOD1*), FUS (for fused in sarcoma) or TLS (translocation in liposarcoma) (*FUS*/*TLS*), and *TARDBP* encoding TAR DNA-binding protein 43 (TDP-43) (Campos-Melo et al. 2013).

miR-206 is highly upregulated in the muscle of the G93A-SOD1 transgenic mice, a well-recognized mouse model of ALS (Gurney et al. 1994; Son et al. 2007; Williams et al. 2009). In contrast, miR-1 and miR-133 expression is dramatically

downregulated (Williams et al. 2009). Upregulation of miR-206 coincides with the progression of the disease, suggesting that it might be involved in ALS disease pathology (Williams et al. 2009). More importantly, absence of miR-206 in the ALS (G93A-SOD1) mouse results in an acceleration of the initiation of symptoms and a decrease in survival of the ALS mice (Fig. 4a–c) (Williams et al. 2009). These findings indicate that miR-206 protects against ALS and the upregulation of miR-206 expression is required to delay the onset of ALS.

In normal muscle, miR-206 expression is enriched in the neuromuscular junctions (NMJ) region of the muscle fibers, which connects motor neurons to muscle fibers (Williams et al. 2009). miR-206 promotes the regeneration of NMJ upon denervation by repressing histone deacetylase 4 (HDAC4), which inhibits nerve reinnervation by inhibiting expression of fibroblast growth factor-binding protein 1 (FGFBP1) (Fig. 4d) (Williams et al. 2009). FGFBP1 is secreted from muscle and can promote innervation by activating FGF proteins on distal motor neuron (Williams et al. 2009). Mice deficient of miR-206 showed significant delay in reinnervation of NMJs in response to denervation. miR-206 serves as a sensor of motor innervation and regulates a retrograde signaling pathway required for the nerve–muscle interactions. The same mechanism is believed to contribute to the earlier disease onset in ALS (G93A-SOD1) mice lacking miR-206. Therefore, the discovery of miR-206 as a modifier of ALS reveals an unappreciated role of muscle-derived factors in the pathogenesis of ALS and suggests the applicability of miRNA-mediated therapy for ALS.

Since the discovery of miR-206 as a modifier of ALS disease pathology, a large group of miRNAs has been identified as being differentially expressed in brain, spinal cord, and peripheral monocytes in ALS mouse models (Butovsky et al. 2012; Campos-Melo et al. 2013; Koval et al. 2013; Shinde et al. 2013). Among them, miR-155 is significantly upregulated in spinal cord tissue of both ALS mice and human patients. Treatment of G93A-SOD1 mice with anti-miR-155 significantly extends survival by 10 days and disease duration by 15 days (Koval et al. 2013). This result indicates that miR-155 is a promising new therapeutic target for human ALS. It remains to be determined whether additional miRNAs can also influence ALS disease onset.

5 miRNAs in Rhabdomyosarcoma

Rhabdomyosarcomas (RMS) are the most common soft tissue sarcomas in children and young adults (Wachtel et al. 2006). It can occur in many places in the body. The most common sites are the structures of the head and neck, the urogenital tract, and the arms or legs. Although the cure rate for patients with localized disease is around 70 %, the presence of metastasis is associated with a much poorer prognosis (Breneman et al. 2003; Oberlin et al. 2008). In addition, current treatment strategies confer significant morbidity and less toxic treatments are urgently needed (Stevens 2005). The defining characteristic of RMS is expression of myogenic differentiation

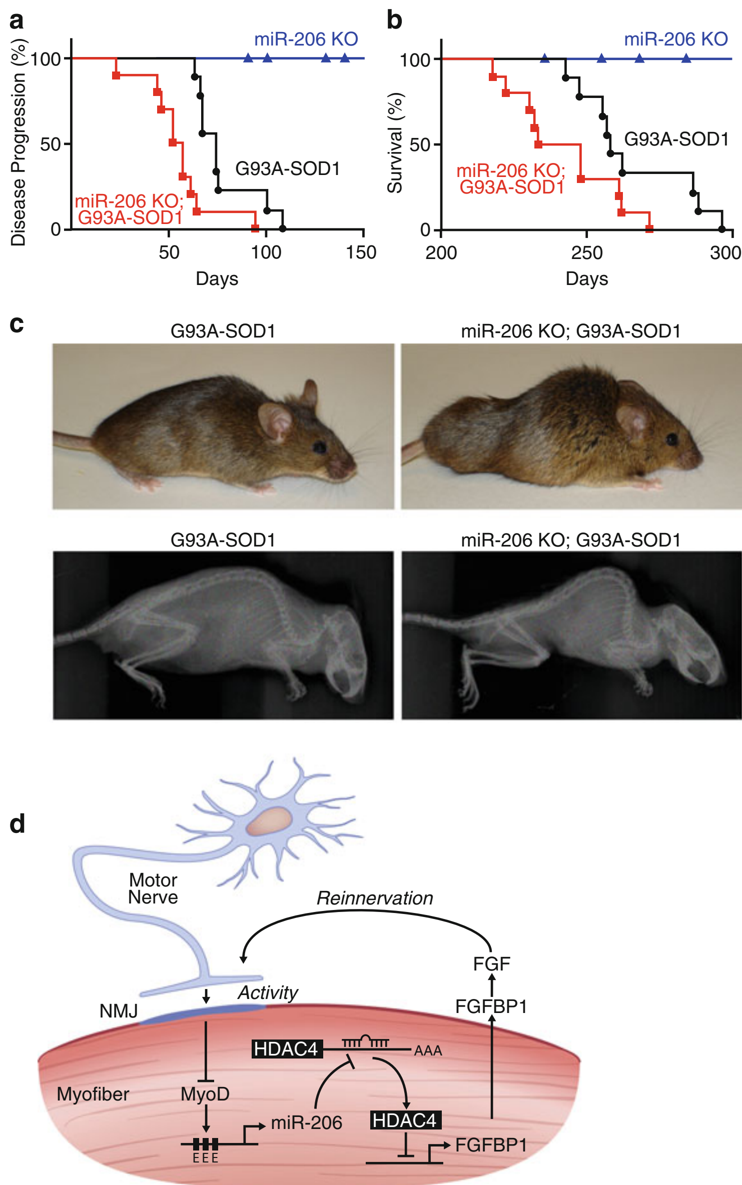


Fig. 4 Regulation of ALS pathogenesis by miR-206. (**a**) Days of disease progression for G93A-SOD1 and miR-206$^{-/-}$;G93A-SOD1 littermates. $P < 0.005$, long-rank test. (**b**) Survival curve for G93A-SOD1 and miR-206$^{-/-}$;G93A-SOD1 littermates. $P < 0.05$, long-rank test. (**c**) G93A-SOD1 and miR-206$^{-/-}$;G93A-SOD1 mice at 7.5 months of age. There is severe atrophy of hindlimb muscles in miR-206$^{-/-}$;G93A-SOD1 mouse. X-ray reveals kyphosis in miR-206$^{-/-}$;G93A-SOD1 mice. (**d**) Model of miR-206-dependent reinnervation of neuromuscular junctions (NMJs)

markers (Merlino and Helman 1999; Wachtel et al. 2006). Although the exact etiology of RMS is unknown, based on the expression of myogenic differentiation markers, such as MyoD and desmin, it is surmised that the cell of origin is a myogenic progenitor cell that failed to undergo terminal differentiation.

Expression of miR-206 and miR-1 is suppressed in primary RMS and RMS cell lines (Taulli et al. 2009; Yan et al. 2009). Forced overexpression of miR-206 in RMS cells promotes myogenic differentiation and blocks tumor growth in xenografted mice (Taulli et al. 2009; Yan et al. 2009). The action of miR-206 in RMS is postulated to be mediated by its repression of the product of the MET proto-oncogene, the Met tyrosine-kinase receptor, which is overexpressed in RMS and has been implicated in RMS pathogenesis (Taulli et al. 2009; Yan et al. 2009). Furthermore, measuring miR-206 levels in RMS samples showed that miR-206 expression levels correlated with clinical behavior of RMS patients, implying a therapeutic potential of miR-206 in treatment of RMS (Missiaglia et al. 2010). Low levels of miR-206 correlated with poor overall survival in metastatic embryonal and alveolar cases without Pax3/7-FOXO1 fusion genes. Similar to miR-206 and miR-1, the level of miR-133a is also dramatically reduced in RMS cell lines. Both miR-1 and miR-133a can inhibit the proliferation of RMS cell lines and exert a strong pro-myogenic influence on these poorly differentiated tumor cells (Rao et al. 2010). In addition, expression of miR-29 is silenced in RMS cells and primary tumors. Reconstitution of miR-29 in RMS in mice inhibits tumor growth and stimulates differentiation, suggesting that miR-29 acts as a tumor suppressor through its pro-myogenic function (Wang et al. 2008).

6 miRNA Modulation as a Novel Therapeutic Approach

miRNAs play key roles in various muscle diseases. Therefore, modulating miRNA expression in vivo could provide a novel method for therapeutic intervention. To date, there are several tools available to selectively modulate miRNA levels in vivo. Antisense based miRNA inhibitors can reduce the levels of pathogenic or aberrantly expressed miRNAs (van Rooij et al. 2008a). Conversely, miRNA mimics can serve to elevate the levels of miRNAs with salutary functions (van Rooij et al. 2008a). The primary effect of a miRNA inhibitor is activation of gene expression and a miRNA mimic is suppression of gene expression.

Antisense Based miRNA Inhibitors For miRNAs whose upregulation in a disease state plays a causal role in the disease, specific inhibition of the miRNA would be therapeutically desirable. This approach aims to inhibit miRNAs by using oligonucleotide with complementarity to endogenous miRNA. The synthetic reverse complement oligonucleotide approach can theoretically act at multiple levels to affect miRNA levels: (1) by binding to the mature miRNA within the RISC and acting as a competitive inhibitor; (2) by binding to the pre-miRNA and preventing its processing or entry into the RISC; (3) by interfering with the

processing or export of the pre- or pri-miRNA from the nucleus (van Rooij et al. 2008a). In any case, the net result is a reduction in the concentration of a specific miRNA-programmed RISC. These miRNA inhibitors include 2′-O-methyl-modified oligonucleotide (anti-miRs), cholesterol conjugated antisense oligonucleotide (antagomiRs), and oligonucleotides using the locked nucleic acid phosphorothioate chemistry (LNA-antimiR). The efficacy of antagomiRs and LNA-antimiRs in silencing individual miRNAs has been demonstrated in various tissues in mice. F or example, antagomiRs against miR-133 appeared sufficient to induce significant hypertrophic growth of the heart (Care et al. 2007). Similarly, antagomiRs against miR-29 induced collagen expression in mice (van Rooij et al. 2008b). It was later demonstrated that LNA modification is more superior to cholesterol conjugation as it results in a thermodynamically stronger duplex formation with the target RNA. Indeed, the efficacy of LNA-anti-miR-122 has been demonstrated in treating hepatitis C virus infection in nonhuman primates (Elmen et al. 2008; Lanford et al. 2010) and has been advanced to human clinical trials. The lack of toxicity or histological changes of LNA-antimiRs has made it an attractive therapeutic for diseases associated with miRNA dysregulation.

miRNA Mimics When reduction of specific miRNAs causes a disease state, increase of that miRNA could be a beneficial therapeutic approach. miRNA mimics are synthetic RNA duplexes in which one strand is the mature miRNA sequence (guide strand) and the other stand is complimentary or partially complementary to the mature miRNA sequence. The miRNA mimics approach is much less developed, compared to the anti-miR approaches. However, promising results have been made in several disease models in rodents. For example, local injection of miR-1, miR-206, and miR-133 mimics can accelerate muscle regeneration in a rat skeletal muscle injury model (Nakasa et al. 2010). Injection of miR-29 mimics in mice results in reduced expression of collagens, consistent with miR-29's role in regulating cardiac fibrosis (van Rooij et al. 2008b). Despite these advances, little is known about the long-term efficacy and toxicity of the miRNA mimics in vivo. Nonetheless, this approach represents an attractive means of enhancing miRNA levels for those downregulated during disease.

7 miRNAs as Biomarkers of Skeletal Muscle Disease

miRNAs are being developed as biomarkers for diseases, since they are resistant to ribonucleases rendering them stable in serum. Changes in miRNA expression in the blood may be reflective of disease. Although miRNAs are expressed in specific tissues, their biological role in systemic circulation remains unknown. It was reported that serum miRNA profiling of *mdx* mice revealed a distinct extracellular miRNA signature (dystromiRs) (Roberts et al. 2013). There was an increase of 57 circulating miRNAs in *mdx* mice compared to wild-type controls, including miR-1, miR-133a, and miR-206. Furthermore, they showed that the levels of

circulating miRNAs followed the development of the underlying muscle pathology in the *mdx* mouse. They also showed that miR-1 levels were increased in the serum of wild-type mice following muscle injury by cardiotoxin, suggesting that high levels of circulating miR-1 are associated with muscle degeneration and injury. Although these studies are tantalizing for the use of circulating miRNAs as biomarkers for muscle disorders, no clinical studies in patients have been reported.

8 Long Noncoding RNAs (lncRNAs) in Skeletal Muscle

As opposed to small noncoding RNAs (such as miRNAs), long noncoding RNAs (lncRNAs) are broadly classified as transcripts longer than 200 nucleotides that have no significant protein coding potential. Like most protein-coding mRNAs, lncRNAs are 5′-capped and polyadenylated (Batista and Chang 2013). It has now been recognized that the mammalian genomes produce thousands of lncRNAs, far exceeding the number of mRNAs (Guttman et al. 2009; Cabili et al. 2011). lncRNAs are typically less evolutionarily conserved, but their expression is strikingly tissue-specific compared to coding genes (Guttman et al. 2009; Cabili et al. 2011). lncRNAs regulate gene expression by diverse mechanisms at the sites of both transcription and translation. At the level of transcription, lncRNAs can bind and titrate away transcription factors from their target chromosomal regions (Rinn and Chang 2012; Batista and Chang 2013). They may act as scaffolds and guide to recruit chromatin modification enzymes to the site of transcription. Such lncRNA guidance can also be exerted through chromosome looping in an enhancer-like mode to repress or activate gene expression (Rinn and Chang 2012; Batista and Chang 2013). At the translation level, lncRNAs can bind to mRNA to regulate mRNA degradation/stability or to promote or inhibit its translation (Rinn and Chang 2012; Batista and Chang 2013). lncRNAs can also act as competitive endogenous RNA (ceRNA) for miRNA, releasing target mRNAs from miRNA-mediated translational repression (Cesana et al. 2011). Considering the diverse functions of lncRNAs in gene expression, it is not surprising that lncRNAs play key roles in diverse cellular processes, and more importantly, in human diseases.

The functions of lnRNAs in muscle development and muscle diseases have just begun to be appreciated. Linc-MD1, a muscle-specific lncRNA cotranscribed with miR-133b, controls myoblast differentiation by acting as ceRNA to inhibit the functions of miR-133 and miR-135 (Cesana et al. 2011). A myostatin-regulated lncRNA, Malat1, is upregulated during myoblast differentiation into myotubes (Watts et al. 2013). Depletion of Malat1 expression in cells suppressed myoblast proliferation. Braveheart, a heart-associated lncRNA, is required for cardiovascular lineage commitment from mesoderm and maintenance of the cardiac fate in neonatal cardiomyocytes, by acting upstream of mesoderm posterior 1 (MesP1) (Klattenhoff et al. 2013). More importantly, lncRNAs have been associated with muscle disorders. In FSHD patients, deletion of D4Z4 repeats in the 4q35 genomic region results in expression of DBE-T, a chromatin-associated lncRNA, which at

sufficient levels can lead to improper establishment of active chromatin and expression of genes from 4q35 (Cabianca et al. 2012). The identification of DBE-T lncRNA in FSHD patients advanced our understanding of the molecular mechanisms and epigenetic switches of FSHD disease progression. Interestingly, multiple novel lncRNAs were identified in the introns of the dystrophin gene (Bovolenta et al. 2012). Forced expression of these lncRNAs in cells causes a negative effect on the expression of endogenous dystrophin gene, indicating that these lncRNAs may contribute to dystrophin expression and disease progression in DMD patients.

To date, our knowledge on the biogenesis and function of lncRNAs is still very limited. However, the genome-scale discovery and characterization of lncRNAs have made it possible to study the functions of individual lncRNAs in the setting of development and human disease. lncRNAs have enormous regulatory potential of gene expression, making them new therapeutic targets for disease intervention.

Concluding Remarks

Skeletal muscle miRNAs are emerging as key players in regulating skeletal muscle development and disease. It is interesting that the same transcription factors that activate protein-coding genes involved in muscle function also regulate miRNAs, suggesting an interconnected relationship and network among muscle-specific miRNAs, transcription factors, and mRNAs. Alteration of miRNA expression is seen in response to physiological or pathological stress, suggesting that miRNAs function to exacerbate or protect muscle during stress or disease. In fact, dysregulation of miRNAs is seen in myopathies, in chronic diseases associated with muscle atrophy as well as with ageing. These observations suggest that miRNAs play an important role in muscle adaptation and maladaptation. To understand how miRNAs function in skeletal muscle biology, further studies are needed to identify target mRNAs and to determine protein expression inhibition. With the availability of this information new regulatory pathways and networks determining muscle biology will emerge. In addition, the availability of miRNA inhibitors and mimics offer approaches to modulate miRNA expression levels with the ultimate goal of providing new therapeutic treatment to combat skeletal muscle disease.

References

Agrawal PB, Greenleaf RS, Tomczak KK, Lehtokari VL, Wallgren-Pettersson C, Wallefeld W, Laing NG, Darras BT, Maciver SK, Dormitzer PR, Beggs AH (2007) Nemaline myopathy with minicores caused by mutation of the CFL2 gene encoding the skeletal muscle actin-binding protein, cofilin-2. Am J Hum Genet 80(1):162–167

Alexander MS, Casar JC, Motohashi N, Myers JA, Eisenberg I, Gonzalez RT, Estrella EA, Kang PB, Kawahara G, Kunkel LM (2011) Regulation of DMD pathology by an ankyrin-encoded miRNA. Skelet Muscle 1:27

Alexander MS, Kawahara G, Motohashi N, Casar JC, Eisenberg I, Myers JA, Gasperini MJ, Estrella EA, Kho AT, Mitsuhashi S, Shapiro F, Kang PB, Kunkel LM (2013) MicroRNA-199a is induced in dystrophic muscle and affects WNT signaling, cell proliferation, and myogenic differentiation. Cell Death Differ 20(9):1194–1208

Alvarez-Saavedra E, Horvitz HR (2010) Many families of C. elegans microRNAs are not essential for development or viability. Curr Biol 20(4):367–373

Ambros V (2010) MicroRNAs: genetically sensitized worms reveal new secrets. Curr Biol 20(14): R598–600

Anderson C, Catoe H, Werner R (2006) MIR-206 regulates connexin43 expression during skeletal muscle development. Nucleic Acids Res 34(20):5863–5871

Aoi W, Naito Y, Mizushima K, Takanami Y, Kawai Y, Ichikawa H, Yoshikawa T (2010) The microRNA miR-696 regulates PGC-1{alpha} in mouse skeletal muscle in response to physical activity. Am J Physiol Endocrinol Metab 298(4):E799–806

Ardite E, Perdiguero E, Vidal B, Gutarra S, Serrano AL, Munoz-Canoves P (2012) PAI-1-regulated miR-21 defines a novel age-associated fibrogenic pathway in muscular dystrophy. J Cell Biol 196(1):163–175

Bartel DP (2004) MicroRNAs: genomics, biogenesis, mechanism, and function. Cell 116(2):281–297

Bartel DP (2009) MicroRNAs: target recognition and regulatory functions. Cell 136(2):215–233

Batista PJ, Chang HY (2013) Long noncoding RNAs: cellular address codes in development and disease. Cell 152(6):1298–1307

Blake DJ, Weir A, Newey SE, Davies KE (2002) Function and genetics of dystrophin and dystrophin-related proteins in muscle. Physiol Rev 82(2):291–329

Bovolenta M, Erriquez D, Valli E, Brioschi S, Scotton C, Neri M, Falzarano MS, Gherardi S, Fabris M, Rimessi P, Gualandi F, Perini G, Ferlini A (2012) The DMD locus harbours multiple long non-coding RNAs which orchestrate and control transcription of muscle dystrophin mRNA isoforms. PLoS One 7(9):e45328

Brack AS, Rando TA (2012) Tissue-specific stem cells: lessons from the skeletal muscle satellite cell. Cell Stem Cell 10(5):504–514

Breneman JC, Lyden E, Pappo AS, Link MP, Anderson JR, Parham DM, Qualman SJ, Wharam MD, Donaldson SS, Maurer HM, Meyer WH, Baker KS, Paidas CN, Crist WM (2003) Prognostic factors and clinical outcomes in children and adolescents with metastatic rhabdomyosarcoma–a report from the Intergroup Rhabdomyosarcoma Study IV. J Clin Oncol 21(1):78–84

Brenner JL, Jasiewicz KL, Fahley AF, Kemp BJ, Abbott AL (2010) Loss of individual microRNAs causes mutant phenotypes in sensitized genetic backgrounds in C. elegans. Curr Biol 20 (14):1321–1325

Bruijn LI, Miller TM, Cleveland DW (2004) Unraveling the mechanisms involved in motor neuron degeneration in ALS. Annu Rev Neurosci 27:723–749

Buckingham M (2007) Skeletal muscle progenitor cells and the role of Pax genes. C R Biol 330(6–7):530–533

Butovsky O, Siddiqui S, Gabriely G, Lanser AJ, Dake B, Murugaiyan G, Doykan CE, Wu PM, Gali RR, Iyer LK, Lawson R, Berry J, Krichevsky AM, Cudkowicz ME, Weiner HL (2012) Modulating inflammatory monocytes with a unique microRNA gene signature ameliorates murine ALS. J Clin Invest 122(9):3063–3087

Cabianca DS, Casa V, Bodega B, Xynos A, Ginelli E, Tanaka Y, Gabellini D (2012) A long ncRNA links copy number variation to a polycomb/trithorax epigenetic switch in FSHD muscular dystrophy. Cell 149(4):819–831

Cabili MN, Trapnell C, Goff L, Koziol M, Tazon-Vega B, Regev A, Rinn JL (2011) Integrative annotation of human large intergenic noncoding RNAs reveals global properties and specific subclasses. Genes Dev 25(18):1915–1927

Cacchiarelli D, Martone J, Girardi E, Cesana M, Incitti T, Morlando M, Nicoletti C, Santini T, Sthandier O, Barberi L, Auricchio A, Musaro A, Bozzoni I (2010) MicroRNAs involved in

molecular circuitries relevant for the Duchenne muscular dystrophy pathogenesis are controlled by the dystrophin/nNOS pathway. Cell Metab 12(4):341–351

Cacchiarelli D, Incitti T, Martone J, Cesana M, Cazzella V, Santini T, Sthandier O, Bozzoni I (2011) miR-31 modulates dystrophin expression: new implications for Duchenne muscular dystrophy therapy. EMBO Rep 12(2):136–141

Cai X, Hagedorn CH, Cullen BR (2004) Human microRNAs are processed from capped, polyadenylated transcripts that can also function as mRNAs. RNA 10(12):1957–1966

Campos-Melo D, Droppelmann CA, He Z, Volkening K, Strong MJ (2013) Altered microRNA expression profile in Amyotrophic Lateral Sclerosis: a role in the regulation of NFL mRNA levels. Mol Brain 6:26

Care A, Catalucci D, Felicetti F, Bonci D, Addario A, Gallo P, Bang ML, Segnalini P, Gu Y, Dalton ND, Elia L, Latronico MV, Hoydal M, Autore C, Russo MA, Dorn GW 2nd, Ellingsen O, Ruiz-Lozano P, Peterson KL, Croce CM, Peschle C, Condorelli G (2007) MicroRNA-133 controls cardiac hypertrophy. Nat Med 13(5):613–618

Cesana M, Cacchiarelli D, Legnini I, Santini T, Sthandier O, Chinappi M, Tramontano A, Bozzoni I (2011) A long noncoding RNA controls muscle differentiation by functioning as a competing endogenous RNA. Cell 147(2):358–369

Chamberlain JS, Banks GB (2008) The value of mammalian models for Duchenne muscular dystrophy in developing therapeutic strategies. Curr Top Dev Biol 84:431–453

Chamberlain JS, Pearlman JA, Muzny DM, Gibbs RA, Ranier JE, Caskey CT, Reeves AA (1988) Expression of the murine Duchenne muscular dystrophy gene in muscle and brain. Science 239 (4846):1416–1418

Chandrasekharan K, Yoon JH, Xu Y, deVries S, Camboni M, Janssen PM, Varki A, Martin PT (2010) A human-specific deletion in mouse Cmah increases disease severity in the mdx model of Duchenne muscular dystrophy. Sci Transl Med 2(42):42ra54

Charge SB, Rudnicki MA (2004) Cellular and molecular regulation of muscle regeneration. Physiol Rev 84(1):209–238

Chen JF, Mandel EM, Thomson JM, Wu Q, Callis TE, Hammond SM, Conlon FL, Wang DZ (2006) The role of microRNA-1 and microRNA-133 in skeletal muscle proliferation and differentiation. Nat Genet 38(2):228–233

Chen JF, Tao Y, Li J, Deng Z, Yan Z, Xiao X, Wang DZ (2010) microRNA-1 and microRNA-206 regulate skeletal muscle satellite cell proliferation and differentiation by repressing Pax7. J Cell Biol 190(5):867–879

Chendrimada TP, Gregory RI, Kumaraswamy E, Norman J, Cooch N, Nishikura K, Shiekhattar R (2005) TRBP recruits the Dicer complex to Ago2 for microRNA processing and gene silencing. Nature 436(7051):740–744

Cheung TH, Quach NL, Charville GW, Liu L, Park L, Edalati A, Yoo B, Hoang P, Rando TA (2012) Maintenance of muscle stem-cell quiescence by microRNA-489. Nature 482 (7386):524–528

Clop A, Marcq F, Takeda H, Pirottin D, Tordoir X, Bibe B, Bouix J, Caiment F, Elsen JM, Eychenne F, Larzul C, Laville E, Meish F, Milenkovic D, Tobin J, Charlier C, Georges M (2006) A mutation creating a potential illegitimate microRNA target site in the myostatin gene affects muscularity in sheep. Nat Genet 38(7):813–818

Crist CG, Montarras D, Buckingham M (2012) Muscle satellite cells are primed for myogenesis but maintain quiescence with sequestration of Myf5 mRNA targeted by microRNA-31 in mRNP granules. Cell Stem Cell 11(1):118–126

Crook R, Ryberg H, Hutton M, Katz J, Simpson EP, Mitsumoto H, Bowser R, Miller RG, Appel SH, Stephan DA (2007) Whole-genome analysis of sporadic amyotrophic lateral sclerosis. N Engl J Med 357(8):775–788

Dalakas MC (2006) Mechanisms of disease: signaling pathways and immunobiology of inflammatory myopathies. Nat Clin Pract Rheumatol 2(4):219–227

Davies KE, Nowak KJ (2006) Molecular mechanisms of muscular dystrophies: old and new players. Nat Rev Mol Cell Biol 7(10):762–773

Deconinck AE, Rafael JA, Skinner JA, Brown SC, Potter AC, Metzinger L, Watt DJ, Dickson JG, Tinsley JM, Davies KE (1997) Utrophin-dystrophin-deficient mice as a model for Duchenne muscular dystrophy. Cell 90(4):717–727

Denli AM, Tops BB, Plasterk RH, Ketting RF, Hannon GJ (2004) Processing of primary microRNAs by the Microprocessor complex. Nature 432(7014):231–235

Dey BK, Gagan J, Yan Z, Dutta A (2012) miR-26a is required for skeletal muscle differentiation and regeneration in mice. Genes Dev 26(19):2180–2191

Dhawan J, Rando TA (2005) Stem cells in postnatal myogenesis: molecular mechanisms of satellite cell quiescence, activation and replenishment. Trends Cell Biol 15(12):666–673

Drummond MJ, McCarthy JJ, Fry CS, Esser KA, Rasmussen BB (2008) Aging differentially affects human skeletal muscle microRNA expression at rest and after an anabolic stimulus of resistance exercise and essential amino acids. Am J Physiol Endocrinol Metab 295(6):E1333–1340

Dunckley T, Huentelman MJ, Craig DW, Pearson JV, Szelinger S, Joshipura K, Halperin RF, Stamper C, Jensen KR, Letizia D, Hesterlee SE, Pestronk A, Levine T, Bertorini T, Graves MC, Mozaffar T, Jackson CE, Bosch P, McVey A, Dick A, Barohn R, Lomen-Hoerth C, Rosenfeld J, O'Connor T, Zhang K, Crook R, Ryberg H, Hutton M, Katz J, Simpson EP, Mitsumoto H, Bowser R, Miller RG, Appel SH, Stephan DA (2007) Whole-genome analysis of sporadic amyotrophic lateral sclerosis. N Engl J Med 357(8):775–788

Durieux AC, Prudhon B, Guicheney P, Bitoun M (2010) Dynamin 2 and human diseases. J Mol Med 88(4):339–350

Eisenberg I, Eran A, Nishino I, Moggio M, Lamperti C, Amato AA, Lidov HG, Kang PB, North KN, Mitrani-Rosenbaum S, Flanigan KM, Neely LA, Whitney D, Beggs AH, Kohane IS, Kunkel LM (2007) Distinctive patterns of microRNA expression in primary muscular disorders. Proc Natl Acad Sci U S A 104(43):17016–17021

Elmen J, Lindow M, Schutz S, Lawrence M, Petri A, Obad S, Lindholm M, Hedtjarn M, Hansen HF, Berger U, Gullans S, Kearney P, Sarnow P, Straarup EM, Kauppinen S (2008) LNA-mediated microRNA silencing in non-human primates. Nature 452(7189):896–899

Filipowicz W, Bhattacharyya SN, Sonenberg N (2008) Mechanisms of post-transcriptional regulation by microRNAs: are the answers in sight? Nat Rev Genet 9(2):102–114

Gan Z, Rumsey J, Hazen BC, Lai L, Leone TC, Vega RB, Xie H, Conley KE, Auwerx J, Smith SR, Olson EN, Kralli A, Kelly DP (2013) Nuclear receptor/microRNA circuitry links muscle fiber type to energy metabolism. J Clin Invest 123(6):2564–2575

Grady RM, Teng H, Nichol MC, Cunningham JC, Wilkinson RS, Sanes JR (1997) Skeletal and cardiac myopathies in mice lacking utrophin and dystrophin: a model for Duchenne muscular dystrophy. Cell 90(4):729–738

Grady RM, Grange RW, Lau KS, Maimone MM, Nichol MC, Stull JT, Sanes JR (1999) Role for alpha-dystrobrevin in the pathogenesis of dystrophin-dependent muscular dystrophies. Nat Cell Biol 1(4):215–220

Greco S, De Simone M, Colussi C, Zaccagnini G, Fasanaro P, Pescatori M, Cardani R, Perbellini R, Isaia E, Sale P, Meola G, Capogrossi MC, Gaetano C, Martelli F (2009) Common micro-RNA signature in skeletal muscle damage and regeneration induced by Duchenne muscular dystrophy and acute ischemia. FASEB J 23(10):3335–3346

Greco S, Perfetti A, Fasanaro P, Cardani R, Capogrossi MC, Meola G, Martelli F (2012) Deregulated microRNAs in myotonic dystrophy type 2. PLoS One 7(6):e39732

Gregory RI, Yan KP, Amuthan G, Chendrimada T, Doratotaj B, Cooch N, Shiekhattar R (2004) The Microprocessor complex mediates the genesis of microRNAs. Nature 432(7014):235–240

Guo C, Willem M, Werner A, Raivich G, Emerson M, Neyses L, Mayer U (2006) Absence of alpha 7 integrin in dystrophin-deficient mice causes a myopathy similar to Duchenne muscular dystrophy. Hum Mol Genet 15(6):989–998

Gurney ME, Pu H, Chiu AY, Dal Canto MC, Polchow CY, Alexander DD, Caliendo J, Hentati A, Kwon YW, Deng HX et al (1994) Motor neuron degeneration in mice that express a human Cu, Zn superoxide dismutase mutation. Science 264(5166):1772–1775

Guttman M, Amit I, Garber M, French C, Lin MF, Feldser D, Huarte M, Zuk O, Carey BW, Cassady JP, Cabili MN, Jaenisch R, Mikkelsen TS, Jacks T, Hacohen N, Bernstein BE, Kellis M, Regev A, Rinn JL, Lander ES (2009) Chromatin signature reveals over a thousand highly conserved large non-coding RNAs in mammals. Nature 458(7235):223–227

Hirai H, Verma M, Watanabe S, Tastad C, Asakura Y, Asakura A (2010) MyoD regulates apoptosis of myoblasts through microRNA-mediated down-regulation of Pax3. J Cell Biol 191(2):347–365

Hoffman EP, Brown RH Jr, Kunkel LM (1987) Dystrophin: the protein product of the Duchenne muscular dystrophy locus. Cell 51(6):919–928

Jungbluth H, Wallgren-Pettersson C, Laporte J (2008) Centronuclear (myotubular) myopathy. Orphanet J Rare Dis 3:26

Kim HK, Lee YS, Sivaprasad U, Malhotra A, Dutta A (2006) Muscle-specific microRNA miR-206 promotes muscle differentiation. J Cell Biol 174(5):677–687

Kirby TJ, McCarthy JJ (2013) MicroRNAs in skeletal muscle biology and exercise adaptation. Free Radic Biol Med 64:95–105

Klattenhoff CA, Scheuermann JC, Surface LE, Bradley RK, Fields PA, Steinhauser ML, Ding H, Butty VL, Torrey L, Haas S, Abo R, Tabebordbar M, Lee RT, Burge CB, Boyer LA (2013) Braveheart, a long noncoding RNA required for cardiovascular lineage commitment. Cell 152 (3):570–583

Koval ED, Shaner C, Zhang P, du Maine X, Fischer K, Tay J, Chau BN, Wu GF, Miller TM (2013) Method for widespread microRNA-155 inhibition prolongs survival in ALS-model mice. Hum Mol Genet 22(20):4127–4135

Kuang S, Kuroda K, Le Grand F, Rudnicki MA (2007) Asymmetric self-renewal and commitment of satellite stem cells in muscle. Cell 129(5):999–1010

Kwon C, Han Z, Olson EN, Srivastava D (2005) MicroRNA1 influences cardiac differentiation in Drosophila and regulates Notch signaling. Proc Natl Acad Sci U S A 102(52):18986–18991

Lanford RE, Hildebrandt-Eriksen ES, Petri A, Persson R, Lindow M, Munk ME, Kauppinen S, Orum H (2010) Therapeutic silencing of microRNA-122 in primates with chronic hepatitis C virus infection. Science 327(5962):198–201

Lee Y, Ahn C, Han J, Choi H, Kim J, Yim J, Lee J, Provost P, Radmark O, Kim S, Kim VN (2003) The nuclear RNase III Drosha initiates microRNA processing. Nature 425(6956):415–419

Lepper C, Partridge TA, Fan CM (2011) An absolute requirement for Pax7-positive satellite cells in acute injury-induced skeletal muscle regeneration. Development 138(17):3639–3646

Liquori CL, Ricker K, Moseley ML, Jacobsen JF, Kress W, Naylor SL, Day JW, Ranum LP (2001) Myotonic dystrophy type 2 caused by a CCTG expansion in intron 1 of ZNF9. Science 293 (5531):864–867

Liu N, Olson EN (2010) MicroRNA regulatory networks in cardiovascular development. Dev Cell 18(4):510–525

Liu N, Williams AH, Kim Y, McAnally J, Bezprozvannaya S, Sutherland LB, Richardson JA, Bassel-Duby R, Olson EN (2007) An intragenic MEF2-dependent enhancer directs muscle-specific expression of microRNAs 1 and 133. Proc Natl Acad Sci U S A 104(52):20844–20849

Liu N, Bezprozvannaya S, Shelton JM, Frisard MI, Hulver MW, McMillan RP, Wu Y, Voelker KA, Grange RW, Richardson JA, Bassel-Duby R, Olson EN (2011) Mice lacking microRNA 133a develop dynamin 2-dependent centronuclear myopathy. J Clin Invest 121(8):3258–3268

Liu N, Williams AH, Maxeiner JM, Bezprozvannaya S, Shelton JM, Richardson JA, Bassel-Duby R, Olson EN (2012) microRNA-206 promotes skeletal muscle regeneration and delays progression of Duchenne muscular dystrophy in mice. J Clin Invest 122(6):2054–2065

Maciotta S, Meregalli M, Cassinelli L, Parolini D, Farini A, Fraro GD, Gandolfi F, Forcato M, Ferrari S, Gabellini D, Bicciato S, Cossu G, Torrente Y (2012) Hmgb3 is regulated by microRNA-206 during muscle regeneration. PLoS One 7(8):e43464

McCarthy JJ, Esser KA (2007) MicroRNA-1 and microRNA-133a expression are decreased during skeletal muscle hypertrophy. J Appl Physiol 102(1):306–313

McCarthy JJ, Mula J, Miyazaki M, Erfani R, Garrison K, Farooqui AB, Srikuea R, Lawson BA, Grimes B, Keller C, Van Zant G, Campbell KS, Esser KA, Dupont-Versteegden EE, Peterson CA (2011) Effective fiber hypertrophy in satellite cell-depleted skeletal muscle. Development 138(17):3657–3666

McNally EM, Pytel P (2007) Muscle diseases: the muscular dystrophies. Annu Rev Pathol 2:87–109

Merlino G, Helman LJ (1999) Rhabdomyosarcoma–working out the pathways. Oncogene 18 (38):5340–5348

Mishima Y, Abreu-Goodger C, Staton AA, Stahlhut C, Shou C, Cheng C, Gerstein M, Enright AJ, Giraldez AJ (2009) Zebrafish miR-1 and miR-133 shape muscle gene expression and regulate sarcomeric actin organization. Genes Dev 23(5):619–632

Missiaglia E, Shepherd CJ, Patel S, Thway K, Pierron G, Pritchard-Jones K, Renard M, Sciot R, Rao P, Oberlin O, Delattre O, Shipley J (2010) MicroRNA-206 expression levels correlate with clinical behaviour of rhabdomyosarcomas. Br J Cancer 102(12):1769–1777

Murphy MM, Lawson JA, Mathew SJ, Hutcheson DA, Kardon G (2011) Satellite cells, connective tissue fibroblasts and their interactions are crucial for muscle regeneration. Development 138 (17):3625–3637

Nakasa T, Ishikawa M, Shi M, Shibuya H, Adachi N, Ochi M (2010) Acceleration of muscle regeneration by local injection of muscle-specific microRNAs in rat skeletal muscle injury model. J Cell Mol Med 14(10):2495–2505

Nance JR, Dowling JJ, Gibbs EM, Bonnemann CG (2012) Congenital myopathies: an update. Curr Neurol Neurosci Rep 12(2):165–174

O'Rourke JR, Georges SA, Seay HR, Tapscott SJ, McManus MT, Goldhamer DJ, Swanson MS, Harfe BD (2007) Essential role for Dicer during skeletal muscle development. Dev Biol 311 (2):359–368

Oberlin O, Rey A, Lyden E, Bisogno G, Stevens MC, Meyer WH, Carli M, Anderson JR (2008) Prognostic factors in metastatic rhabdomyosarcomas: results of a pooled analysis from United States and European cooperative groups. J Clin Oncol 26(14):2384–2389

Perbellini R, Greco S, Sarra-Ferraris G, Cardani R, Capogrossi MC, Meola G, Martelli F (2011) Dysregulation and cellular mislocalization of specific miRNAs in myotonic dystrophy type 1. Neuromuscul Disord 21(2):81–88

Quiat D, Voekler K, Pei J, Grishin N, Bassel-Duby R, Olson EN (2011) Concerted regulation of myofiber specific gene expression and muscle performance by the transcriptional repressor Sox6. Proc Natl Acad Sci U S A 108:10196–201

Rao PK, Kumar RM, Farkhondeh M, Baskerville S, Lodish HF (2006) Myogenic factors that regulate expression of muscle-specific microRNAs. Proc Natl Acad Sci U S A 103(23):8721–8726

Rao PK, Missiaglia E, Shields L, Hyde G, Yuan B, Shepherd CJ, Shipley J, Lodish HF (2010) Distinct roles for miR-1 and miR-133a in the proliferation and differentiation of rhabdomyosarcoma cells. FASEB J 24(9):3427–3437

Rinn JL, Chang HY (2012) Genome regulation by long noncoding RNAs. Annu Rev Biochem 81:145–166

Roberts TC, Godfrey C, McClorey G, Vader P, Briggs D, Gardiner C, Aoki Y, Sargent I, Morgan JE, Wood MJ (2013) Extracellular microRNAs are dynamic non-vesicular biomarkers of muscle turnover. Nucleic Acids Res 41(20):9500–9513

Romero NB (2010) Centronuclear myopathies: a widening concept. Neuromuscul Disord 20 (4):223–228

Rosenberg MI, Georges SA, Asawachaicharn A, Analau E, Tapscott SJ (2006) MyoD inhibits Fstl1 and Utrn expression by inducing transcription of miR-206. J Cell Biol 175(1):77–85

Safdar A, Abadi A, Akhtar M, Hettinga BP, Tarnopolsky MA (2009) miRNA in the regulation of skeletal muscle adaptation to acute endurance exercise in C57Bl/6J male mice. PLoS One 4(5): e5610

Sambasivan R, Yao R, Kissenpfennig A, Van Wittenberghe L, Paldi A, Gayraud-Morel B, Guenou H, Malissen B, Tajbakhsh S, Galy A (2011) Pax7-expressing satellite cells are indispensable for adult skeletal muscle regeneration. Development 138(17):3647–3656

Shinde S, Arora N, Bhadra U (2013) A Complex Network of MicroRNAs Expressed in Brain and Genes Associated with Amyotrophic Lateral Sclerosis. Int J Genomics 2013:383024

Small EM, Olson EN (2011) Pervasive roles of microRNAs in cardiovascular biology. Nature 469 (7330):336–342

Sokol NS, Ambros V (2005) Mesodermally expressed Drosophila microRNA-1 is regulated by Twist and is required in muscles during larval growth. Genes Dev 19(19):2343–2354

Son M, Puttaparthi K, Kawamata H, Rajendran B, Boyer PJ, Manfredi G, Elliott JL (2007) Overexpression of CCS in G93A-SOD1 mice leads to accelerated neurological deficits with severe mitochondrial pathology. Proc Natl Acad Sci U S A 104(14):6072–6077

Stevens MC (2005) Treatment for childhood rhabdomyosarcoma: the cost of cure. Lancet Oncol 6 (2):77–84

Taulli R, Bersani F, Foglizzo V, Linari A, Vigna E, Ladanyi M, Tuschl T, Ponzetto C (2009) The muscle-specific microRNA miR-206 blocks human rhabdomyosarcoma growth in xenotransplanted mice by promoting myogenic differentiation. J Clin Invest 119(8):2366–2378

Tedesco FS, Dellavalle A, Diaz-Manera J, Messina G, Cossu G (2010) Repairing skeletal muscle: regenerative potential of skeletal muscle stem cells. J Clin Invest 120(1):11–19

van Rooij E, Marshall WS, Olson EN (2008a) Toward microRNA-based therapeutics for heart disease: the sense in antisense. Circ Res 103(9):919–928

van Rooij E, Sutherland LB, Thatcher JE, DiMaio JM, Naseem RH, Marshall WS, Hill JA, Olson EN (2008b) Dysregulation of microRNAs after myocardial infarction reveals a role of miR-29 in cardiac fibrosis. Proc Natl Acad Sci U S A 105(35):13027–13032

van Rooij E, Quiat D, Johnson BA, Sutherland LB, Qi X, Richardson JA, Kelm RJ Jr, Olson EN (2009) A family of microRNAs encoded by myosin genes governs myosin expression and muscle performance. Dev Cell 17(5):662–673

von Maltzahn J, Jones AE, Parks RJ, Rudnicki MA (2013) Pax7 is critical for the normal function of satellite cells in adult skeletal muscle. Proc Natl Acad Sci U S A 110(41):16474–16479

Wachtel M, Runge T, Leuschner I, Stegmaier S, Koscielniak E, Treuner J, Odermatt B, Behnke S, Niggli FK, Schafer BW (2006) Subtype and prognostic classification of rhabdomyosarcoma by immunohistochemistry. J Clin Oncol 24(5):816–822

Wallace GQ, McNally EM (2009) Mechanisms of muscle degeneration, regeneration, and repair in the muscular dystrophies. Annu Rev Physiol 71:37–57

Wang YX, Zhang CL, Yu RT, Cho HK, Nelson MC, Bayuga-Ocampo CR, Ham J, Kang H, Evans RM (2004) Regulation of muscle fiber type and running endurance by PPARdelta. PLoS Biol 2 (10):e294

Wang H, Garzon R, Sun H, Ladner KJ, Singh R, Dahlman J, Cheng A, Hall BM, Qualman SJ, Chandler DS, Croce CM, Guttridge DC (2008) NF-kappaB-YY1-miR-29 regulatory circuitry in skeletal myogenesis and rhabdomyosarcoma. Cancer Cell 14(5):369–381

Wang L, Zhou L, Jiang P, Lu L, Chen X, Lan H, Guttridge DC, Sun H, Wang H (2012) Loss of miR-29 in myoblasts contributes to dystrophic muscle pathogenesis. Mol Ther 20(6):1222–1233

Watts R, Johnsen VL, Shearer J, Hittel DS (2013) Myostatin-induced inhibition of the long noncoding RNA Malat1 is associated with decreased myogenesis. Am J Physiol Cell Physiol 304(10):C995–1001

Williams AH, Valdez G, Moresi V, Qi X, McAnally J, Elliott JL, Bassel-Duby R, Sanes JR, Olson EN (2009) MicroRNA-206 delays ALS progression and promotes regeneration of neuromuscular synapses in mice. Science 326(5959):1549–1554

Yan D, Dong Xda E, Chen X, Wang L, Lu C, Wang J, Qu J, Tu L (2009) MicroRNA-1/206 targets c-Met and inhibits rhabdomyosarcoma development. J Biol Chem 284(43):29596–29604

Yin H, Price F, Rudnicki MA (2013) Satellite cells and the muscle stem cell niche. Physiol Rev 93 (1):23–67

Zacharewicz E, Lamon S, Russell AP (2013) MicroRNAs in skeletal muscle and their regulation with exercise, ageing, and disease. Front Physiol 4:266

Zhao Y, Samal E, Srivastava D (2005) Serum response factor regulates a muscle-specific microRNA that targets Hand2 during cardiogenesis. Nature 436(7048):214–220

Adult Skeletal Muscle Stem Cells

Ramkumar Sambasivan and Shahragim Tajbakhsh

Abstract Skeletal muscles in vertebrates have a phenomenal regenerative capacity. A muscle that has been crushed can regenerate fully both structurally and functionally within a month. Remarkably, efficient regeneration continues to occur following repeated injuries. Thousands of muscle precursor cells are needed to accomplish regeneration following acute injury. The differentiated muscle cells, the multinucleated contractile myofibers, are terminally withdrawn from mitosis. The source of the regenerative precursors is the skeletal muscle stem cells—the mononucleated cells closely associated with myofibers, which are known as satellite cells. Satellite cells are mitotically quiescent or slow-cycling, committed to myogenesis, but undifferentiated. Disruption of the niche after muscle damage results in their exit from quiescence and progression towards commitment. They eventually arrest proliferation, differentiate, and fuse to damaged myofibers or make de novo myofibers. Satellite cells are one of the well-studied adult tissue-specific stem cells and have served as an excellent model for investigating adult stem cells. They have also emerged as an important standard in the field of ageing and stem cells. Several recent reviews have highlighted the importance of these cells as a model to understand stem cell biology. This chapter begins with the discovery of satellite cells as skeletal muscle stem cells and their developmental origin. We discuss transcription factors and signalling cues governing stem cell function of satellite cells and heterogeneity in the satellite cell pool. Apart from satellite cells, a number of other stem cells have been shown to make muscle and are being considered as candidate stem cells for amelioration of muscle degenerative diseases. We discuss these "offbeat" muscle stem cells and their status as adult skeletal muscle stem cells vis-a-vis satellite cells. The ageing context is highlighted in the concluding section.

R. Sambasivan (✉)
Institute for Stem Cell Biology and Regenerative Medicine, GKVK, Bellary Road, Bangalore 560065, India
e-mail: ramkumars@instem.res.in

S. Tajbakhsh
Department of Developmental and Stem Cell Biology, CNRS URA 2578, Institut Pasteur, 25 rue du Dr. Roux, 75724 Paris Cedex 15, France
e-mail: shahragim.tajbakhsh@pasteur.fr

© Springer-Verlag Berlin Heidelberg 2015

B. Brand-Saberi (ed.), *Vertebrate Myogenesis*, Results and Problems in Cell Differentiation 56, DOI 10.1007/978-3-662-44608-9_9

1 Discovery of Satellite Cells: Skeletal Muscle Stem Cells

Skeletal muscle tissue is made of long tube-like multinucleated cells called myofibers. The myofibers harbour the specialised actin–myosin apparatus that enables the muscle to perform contractile function generating force for locomotion or maintenance of posture. During development the myofibers are formed by fusion of hundreds of nuclei. Apart from the contractile fibers, the connective tissue, vasculature, and the innervating motor and sensory nerves constitute the muscle tissue. Although the cellular turnover in skeletal muscle is low, the wear and tear caused by the mechanical nature of its function necessitates homeostatic cellular replacement. Importantly, the tissue has a robust regenerative potential. For example, skeletal muscle damaged by injury regenerates within a span of 2–3 weeks to achieve form and function equivalent to the original. The regenerative/repair potential of skeletal muscle was recognized in the nineteenth century (see Scharner and Zammit 2011). Evidence for the existence of skeletal muscle stem cells to enable regeneration came almost a century later. Independently, Alexander Mauro and Bernard Katz noticed mono-nucleated cells in close association with myofibers in frog and rat muscles using electron microscopy (Katz 1961; Mauro 1961). Mauro speculated these cells to be the stem cells of skeletal muscle that help accomplish repair and regeneration. As these cells were outside the plasma membrane of the myofiber, yet contained within the extracellular matrix enclosing the myofiber, these cells were called "satellite cells" (Fig. 1). When single myofibers were isolated and placed in cell culture, the satellite cells migrated out and generated proliferating muscle precursor cells (myoblasts), which then fused to form multinucleated myofibers in vitro (Bischoff 1975; Konigsberg et al. 1975). These and several other similar studies strongly pointed to satellite cells as skeletal muscle stem cells, and this notion had been widely accepted in the field. In a more recent study, single fibers from mouse hindlimb muscles with an average of seven satellite cells were transplanted into injured muscles. The extensive contribution of the small number of donor satellite cells to host muscle tissue as well as to new satellite cells in the regenerated muscle tissue is one of the most convincing evidences in vivo that satellite cells are stem cells (Collins et al. 2005). Transplanted single satellite cells, isolated on the basis of expression of a combination of positive and negative surface markers, also contribute significantly to differentiated muscle cells during muscle regeneration as well as generated new satellite cells establishing stem cell function (Sacco et al. 2008). A stringent measure of stem cell function is the long-term potential to generate differentiated cells as well as self-renew. This capacity has been demonstrated for haematopoietic stem cells by serial transplantations (see Perry and Li 2010). A similar experiment has been done for satellite cells (Rocheteau et al. 2012). A pool of satellite cells isolated by FACS were grafted in injured muscle and allowed to participate in the regeneration process. At the end of 3 weeks, when regeneration was complete, donor satellite cells marked by GFP were sorted again and grafted into injured muscle of another host. This cycle was repeated up to six times successfully. Given the low cellular turnover of the muscle

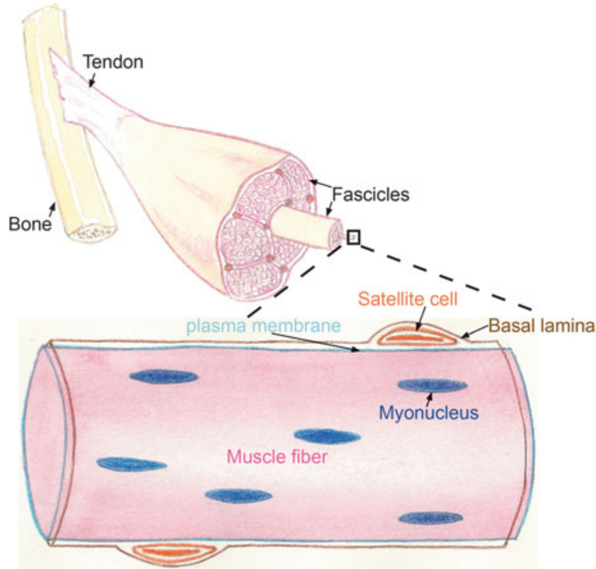


Fig. 1 Anatomical location of satellite cell. Illustration of a skeletal muscle cross-section revealing the "bundles of bundles" organization. Muscles are made of bundles of fascicles, which are in turn bundles of muscle fibers. *Bottom panel* zooms in on a portion of the muscle fibre. The nuclei of the multinucleated muscle fibre, myonuclei, are located in the periphery of the fibre within the plasma membrane. Satellite cells are sandwiched between the plasma membrane of host fibre and the basal lamina of the basement membrane enveloping the fibre

tissue and the extent of cellular loss and replenishment involved in the injury-induced regeneration experiments, this experiment demonstrates robust long-term regenerative capacity of satellite cells and attests to its "stemness". To date, many lines of evidence have been generated by studies indicating that the satellite cells are skeletal muscle stem cells. Some of these studies will be cited in this chapter in different contexts of satellite cell biology.

2 Developmental Origin of Satellite Cells

Most of the studies on satellite cell biology, traditionally, have focussed on limb muscle or trunk muscle stem cells. Using quail–chick chimaera to trace derivatives of donor quail embryonic tissues in chick hosts (Armand et al. 1983), the origin of trunk muscle satellite cells was shown to be the somites. Somites are epithelial segments of mesoderm on either side of the body axis, which harbour the developmental founder muscle stem cell population. Somitic origin of limb muscle satellite cells was also demonstrated by genetic labelling and tracing in chick as well as mice (Schienda et al. 2006; Tajbakhsh 2009; Murphy and Kardon, 2011). Pax3 and Pax7

are paired-box transcription factors that mark developmental muscle progenitors in the somites. Several independent studies, documenting the persistence of a population expressing Pax3 and later Pax7 throughout developmental and post-natal muscle formation, had established the continuity between developmental muscle founder cells and adult muscle satellite cells (Ben-Yair and Kalcheim 2005; Gros et al. 2005; Kassar-Duchossoy et al. 2005; Relaix et al. 2005). Musculature in the head derives from non-somitic cranial mesoderm with a distinct regulatory program (Tajbakhsh et al. 1997). The satellite cells of head muscles also derive from cranial mesoderm (Harel et al. 2009; Sambasivan et al. 2009). Moreover, the satellite cells continue to have a gene expression signature reminiscent of their cranial mesoderm origin. Thus, it appears that all satellite cells share their embryonic origin with their host muscles and the subset of progenitors that assure continued muscle development eventually assume the role of adult stem cells. The contribution to the satellite cell pool from an originally non-muscle progenitor pool has also been reported, but this appears to be minor (Dellavalle et al. 2011). Remarkably, satellite cells isolated from extraocular muscles, a head muscle group governing eye movements, participates effectively in limb muscle regeneration upon heterotopic transplantation (Sambasivan et al. 2009). This suggests that in spite of varied origin, satellite cells of all muscles have universal potential to effect myogenic stem cell function.

3 Regulatory Mechanisms Governing Satellite Cell Function

Unlike blood, skin, or intestine, skeletal muscle tissue is reported to exhibit low cellular turnover. The average age of intercostal muscle fibers in humans is estimated to be 15 years (Spalding et al. 2005). Hence, in adult homeostatic muscle, constant high stem cell activity is not required. As the nuclear addition to the growing muscle ceases in juveniles, satellite cells enter a mitotically quiescent G_0 state in adult muscles (White et al. 2010). When the muscle tissue needs repair or an injury warrants a regenerative response, satellite cells are awakened from quiescence. They proliferate, commensurate to the extent of repair/regenerative demand, commit further to muscle lineage becoming myoblasts, cease expansion, differentiate and either fuse with damaged muscle fibre or make new myofibers to restore the muscle structure and function (Fig. 2). Inflammation plays a key role in the regenerative response (see Kharraz et al. 2010) and connective tissue, vasculature as well as innervation ought to be restored in a coordinated fashion for functional regeneration of the tissue. Globally, at the cellular and molecular level myofiber regeneration largely recapitulates muscle lineage progression during development (Fig. 2). Here, we will focus on skeletal muscle stem cell function and highlight the role of transcription factors and signalling cues that regulate generation and maintenance of quiescent stem cell state of satellite cells and their self-renewal.

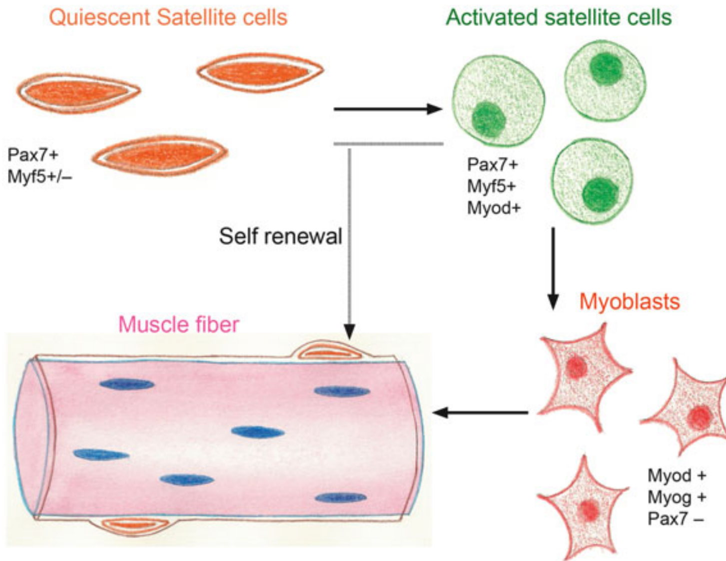


Fig. 2 Lineage progression during muscle differentiation. During muscle regeneration, mitotically quiescent satellite cells are activated. All satellite cells express Pax7, whereas the muscle regulatory factor Myf5 protein expression is seen in a proportion of satellite cells. The activated progenitors proliferate; they continue to express the satellite cell marker Pax7, but also induce *Myod* expression and are referred to as myoblasts. When Myog is induced, they commit to differentiate. Myoblasts fuse with each other to make the muscle fibre. The *gray-line arrow* points to self-renewal. The precise regulatory state of the cells that renew satellite cell pool in vivo is not known. Renewal is possibly accomplished mainly by the progenitor population

Both in vitro and in vivo models have helped understand regeneration and satellite cell function. The de novo formation of myofibers from myoblasts in vitro, and undifferentiated stem-like "reserve cells" formed in differentiating cultures (Kitzmann et al. 1998) have been used as a model system. In vivo, hindlimb muscles of mice, especially, the easy-to-access *Tibialis anterior*, are injured using a range of "traumatic" insults such as myotoxic snake venoms (notexin, cardiotoxin), glycerol, barium chloride, or physical injury by placing a cryofrozen metal rod over exposed muscle for studying satellite cell behaviour/function. Strenuous exercise on a running wheel and swimming are "physiological" injury methods, wherein several muscle groups could be studied (Gayraud-Morel et al. 2009).

Pulse-chase experiments to detect DNA synthesis by thymidine analogue incorporation found satellite cells to be quiescent and activated to proliferate upon muscle injury (Snow 1977). Quiescent satellite cells express Pax7, arguably the most reliable marker to identify muscle stem cells from mid-embryogenesis to aged mice (Tajbakhsh 2009). *Pax7* is required for generation of satellite cells as *Pax7* null muscle is almost devoid of satellite cells (Seale et al. 2000). *Pax7* null mice are considerably smaller and this could at least in part be due to failure of muscle

growth, although craniofacial abnormalities and defects in the central nervous system could also contribute to this phenotype (Mansouri et al. 1996). In muscle, accelerated differentiation as well as apoptotic loss of satellite cells in the absence of *Pax7* function has been reported (Gunther et al. 2013; Lee et al. 2012; Lepper et al. 2009; Relaix et al. 2006). In the majority of muscles, the paralogue of *Pax7*, *Pax3* is downregulated during foetal development from embryonic day E14.5 (Goulding et al. 1991; Horst et al. 2006) and only a subset of satellite cells express *Pax3* (Montarras et al. 2005). However, *Pax3* fails to rescue satellite cells in *Pax7* mutants (Relaix et al. 2006). Both of these paired-box transcription factors harbour a homeodomain. Pax7 was found to exhibit higher binding affinity to homeodomain-binding sites than Pax3 and this could explain nonredundancy (Soleimani et al. 2012). *Pax7*, however, is not necessary for the specification of juvenile satellite cells as these cells are found in perinatal *Pax7* mutant muscle (Kuang et al. 2006; Lepper et al. 2011; Oustanina et al. 2004; Relaix et al. 2006). Conditional knockout of *Pax7* revealed critical requirement for satellite cells around 2 weeks after birth (Lepper et al. 2011) and indispensability of its function for maintenance of the adult satellite cell pool (Gunther et al. 2013; von Maltzahn et al. 2013). Importantly, inactivation of *Pax7* in adult satellite cells abrogates muscle regeneration (Gunther et al. 2013; von Maltzahn et al. 2013). Pax7 has been shown to recruit histone methyl transferase complex to the regulatory sequences of *Myf5* and thus positively regulate muscle gene expression (McKinnell et al. 2008). A role for Pax7 as a pioneer transcription factor was reported recently in pituitary (Budry et al. 2012), thereby extending the regulatory repertoire of this transcription to chromatin modelling. However, its molecular function in maintenance of quiescent satellite cell pool remains unknown.

Of the four muscle regulatory basic-helix-loop-helix transcription factors (bHLH; MRFs) Myf5, Myod, Mrf4 (Myf6), and Myog (myogenin), only Myf5 is widely expressed in quiescent satellite cells. *Myf5*^{nlacZ} reporter (Beauchamp et al. 2000) as well as Myf5 protein is expressed in satellite cells (Gayraud-Morel et al. 2012). Muscle regeneration in *Myf5* null mutants is deficient, likely due to potential Myf5 requirement in expansion of myoblasts or in balancing self-renewal and differentiation (Gayraud-Morel et al. 2012; Ustanina et al. 2007). *Myf5* may function to help maintain myogenic commitment of satellite cells. However, satellite cells in *Myf5* heterozygous mice are more committed to differentiation (Gayraud-Morel et al. 2012). Thus the molecular function of *Myf5* in quiescent satellite cells remains unclear.

Once satellite cells are activated in response to repair/regenerative stimuli, *Myf5*, *Myod*, and eventually *Myog* are activated to drive differentiation. While some satellite cells induce these factors within a few hours of an activation cue, the majority express these factors a day after the injury. Though Myod protein is not detected in quiescent adult satellite cells, it appears to play a key role in balancing differentiation and stem cell generation during development. In *Myod* null mice differentiation of activated satellite cells is delayed as they continue to proliferate indicating *Myod* function in balancing differentiation and self-renewal (Cornelison et al. 2000; Megeney et al. 1996; White et al. 2000; Yablonka-Reuveni et al. 1999). In transplantation experiments, *Myod* null satellite cells survive better as apoptosis

is suppressed (Asakura et al. 2007). This raises the possibility that under physiological conditions Myod might control the number of myoblasts by regulating cell survival as well. Although *Myog* regulates differentiation of muscle cells and plays a key role in the embryo, it appears to be dispensable for differentiation in the adult (Hasty et al. 1993; Meadows et al. 2008, 2011; Moncaut et al. 2013; Nabeshima et al. 1993).

The cues from the milieu that act in concert with the intrinsic factors discussed above have also been studied extensively. A role of the myofiber in regulating quiescence and activation of satellite cells was revealed by a simple experiment. On isolated single myofibers in culture, satellite cells proliferated better when the myofiber was selectively killed using a toxin (Bischoff 1990). Since then, a role for Notch signalling has been shown in regulating the quiescent state of satellite cells. In fact, conditional removal of *Rbpj*, an effector of Notch, during homeostasis causes loss of quiescent satellite cells. While some are activated and enter the cell cycle, the majority of satellite cells directly differentiate from G_0 without undergoing S-phase (Bjornson et al. 2012; Mourikis et al. 2012b). By contrast, if *Rbpj* activity is abrogated after cell cycle entry, the majority of the myogenic cells undergo S-phase. These findings underscore the diverse contextual roles of Notch signalling during muscle homeostasis and regeneration. Overexpression of NICD, the constitutively active intracellular domain of Notch receptor, in primary myoblasts inhibits DNA synthesis and cell proliferation. In vivo, when overexpressed specifically in satellite cells, NICD increases the number of *Pax7* expressing satellite cells but regeneration is impaired (Wen et al. 2012). This study also showed that Rbpj directly regulates *Pax7* expression. Intriguingly, however, loss of Notch3 receptor results in increased number of satellite cells and mutant muscle mass increases after multiple rounds of muscle injury (Kitamoto and Hanaoka 2010). It has been reported that Notch3 antagonizes Notch1. At this juncture, it is relevant to note that Notch signalling is critical for sustaining the $Pax7^{+}$ muscle progenitor population during development. Loss of Delta like-1 (Dll1; a Notch ligand) accelerates muscle differentiation and premature depletion of somitic muscle progenitors (Schuster-Gossler et al. 2007), whereas NICD overexpression abrogates differentiation resulting in expansion of these progenitors (Mourikis et al. 2012a). Notch is also key for the emergence of satellite cells and occupation of sub-laminar niche (Brohl et al. 2012; Fukada et al. 2011). Though, Notch and Delta signalling has been reported to activate satellite cells upon muscle injury, such a role is unclear given that the loss of function of Rbpj does not compromise transit amplification of myogenic cells (Mourikis and Tajbakhsh 2014). Together, these evidences show that Notch signalling through distinct receptors regulates the maintenance of $Pax7^{+}$ muscle progenitors throughout development, the emergence of undifferentiated quiescent satellite cells postnatally, and maintenance of stem cell state in the adults.

Tie-2/angiopoietin signalling through ERK 1/2 has also been shown to promote renewal of satellite cells by favouring mitotic quiescence and blocking muscle differentiation (Abou-Khalil et al. 2009). In addition, calcitonin signalling has been shown to maintain satellite cells in quiescence. Calcitonin receptor is expressed in a quiescent-specific manner and proliferation of satellite cells is inhibited by elcatonin, an exogenous agonist (Fukada et al. 2007).

Wnt signalling has also been proposed to play a role in maintenance of quiescence. When isolated myofibers are co-cultured with cells expressing *Wnt4* and *Wnt6*, satellite cell proliferation is inhibited (Otto et al. 2008). Moreover, Wnt pathway was shown to be active in quiescent myogenic cells in vitro (Subramaniam et al. 2013). However, recent genetic studies in the mouse have modified our view on the role of Wnt (see below). Wnt through Frizzled receptors activates either the β-catenin pathway or the planar cell polarity (PCP) pathway. Both these pathways are implicated in regulating satellite cell proliferation. The Wnt PCP pathway activated by Wnt7a and Frizzled 7 (Fzd7) plays a role in self-renewing divisions of satellite cells (Le Grand et al. 2009). On single myofibers in culture, recombinant Wnt7a promotes symmetric expansion of satellite cells, whereas silencing *Fzd7* or the planar cell polarity effector Vangl2 had the opposite effect on satellite cell expansion. In mutant mice lacking *Wnt7a*, satellite cell number reduced following a round of experimentally induced regeneration (Le Grand et al. 2009). Thus, Wnt PCP pathway promotes self-renewing cell divisions of satellite cells. The mechanism proposed is that of PCP pathway orienting cytokinesis such that both daughter cells contact the basal lamina. The majority of first divisions observed ex vivo were planar and not all divisions with planar orientation are self-renewing (Kuang et al. 2007). Notably, Sprouty1, an antagonist of FGF pathway is key for activated satellite cells to revert to and maintain quiescence during self-renewal (Shea et al. 2010). Thus, multiple pathways finely control generation and maintenance of stem cell state.

A number of signals are known to activate satellite cells. Following expansion of the satellite cell pool, cessation of proliferation and onset of differentiation must occur to achieve efficient repair/regeneration. One of the early factors identified to activate satellite cells out of quiescence and promote proliferation is the hepatocyte growth factor/scatter factor (Tatsumi et al. 1998). HGF activates the MAPK/ERK signalling pathway via Met receptor tyrosine kinase (Day et al. 1999) and as revealed by studies on cultured muscle cells, suppresses myogenic differentiation to allow expansion of progenitors (Halevy and Cantley 2004; Jones et al. 2001). Proliferation of satellite cells is also supported by cytokines such as fibroblast growth factors (FGFs) and insulin-like growth factors (IGFs). Quenching endogenous basic FGF (FGF2) by injecting specific antibody attenuated myoblast expansion. Activation/proliferation of satellite cells is compromised in *Fgf6* null mutant muscle, which displays reduced numbers of myoblasts and defective regeneration (Floss et al. 1997). Sphingosine-1-phosphate (S1P) is a signalling sphingolipid, derived from the membrane phospholipid sphingomyelin. S1P acts to induce satellite cell proliferation. Exogenous S1P acts a mitogen for quiescent muscle "reserve cells" in culture, whereas inhibition of S1P biosynthesis reduces "reserve cell" proliferation as well as impairs muscle regeneration in vivo (Nagata et al. 2006). Other factors from the niche such as vascular endothelial growth factor (VEGF), brain-derived neurotrophic factor (BDNF), and nitric oxide (NO) also regulate satellite cell activation and expansion. For a comprehensive discussion of the various signalling pathways involved in these processes, see (Yin et al. 2013).

The study that had implicated Wnt4 and 6 on satellite cell quiescence also showed that the β-cat pathway was active in proliferating satellite cells and that

Wnt1, Wnt3a, and 5a promoted expansion of satellite cells (Otto et al. 2008). Furthermore, Wnt signalling has been suggested to control the switch from proliferation to differentiation. Exogenous Wnt3A treated regenerating muscle in mice manifested accelerated differentiation and inhibition of Wnt signalling lead to impaired regeneration (Brack et al. 2008). Notably, muscle regeneration was susceptible to Wnt inhibition only at later stages when myogenic differentiation occurs. The study proposed a late requirement for Wnt, in contrast to early requirement for Notch. Those studies also suggested that activation of GSK3β by Notch and inhibition by Wnt could provide the switch from proliferative phase to differentiation. Thus, these studies suggested that Wnt signalling plays multiple roles in satellite cell biology—in quiescence, self-renewal, proliferation, and differentiation. Surprisingly, however, there is no apparent requirement of Wnt-β-catenin signaling in satellite cell function. Specific abrogation of Wnt-β-catenin signaling in satellite cells has no observable impact on quiescence / self-renewal of satellite cells or on muscle regeneration (Murphy et al. 2014). However, Wnt pathway is active in proliferating satellite cells and might play a role in their expansion. It is worth noting that different Wnts, as discussed above, have different effects on satellite cells (Otto et al. 2008). Furthermore, different levels of Wnt activation have been suggested to produce distinct cell fate output (Subramaniam et al. 2013). It is likely that the diversity in Wnt ligands and/or strength of Wnt pathway activation could underlie the pleiotropic function of Wnt signalling in satellite cell biology. The relationship with the Notch pathway, however, remains elusive given the recent reports that Notch activity is suppressed during the early stages of satellite cell activation (Mourikis et al. 2012b).

Insulin-like growth factors (IGFs) are key signalling molecules that act in the final step of lineage progression of satellite cells. They play a central role in muscle growth by promoting differentiation and hypertrophy. For reviews see (Vinciguerra et al. 2010; Zanou and Gailly 2013).

In essence, a complex regulatory network comprising transcription factors and signalling molecules tightly orchestrate satellite cell function by controlling quiescence, activation, proliferation, and finally differentiation and self-renewal. One of the approaches to ameliorate muscle-wasting diseases is by modulating the pathways regulating satellite cell function. However, more in vivo studies are required to unveil the precise roles of these pathways as in vitro studies have in some cases been misleading. A concerted effort is required to make this extensive knowledge of the regulatory network clinically applicable.

4 Heterogeneity and Asymmetric Divisions in Satellite Cell Population

All satellite cells are not equal. Differences exist in the expression of markers, extent of commitment into the muscle lineage, in the expression of traits associated with stem cells as well as stem cell potential. A number of molecular markers

identify satellite cells. Markers such as CD34, SM/C-2.6, and α7-integrin have been used for enrichment by fluorescence activated cell sorting (FACS) of satellite cells. However, these markers are not unique to satellite cells in the muscle, and hence hematopoietic and endothelial cells ought to be eliminated by using markers such as CD45, Ter111, and CD31 (Tedesco et al. 2010). Human muscle satellite cells express CD56, an isoform of neural cell adhesion molecule, and it is widely used for enrichment by FACS (Illa et al. 1992). Markers such as Met, m-Cadherin, Caveolin1, Calcitonin receptor, sphingomyelin-binding lysenin, Nestin, SM/C-2.6, Syndecan1/4, and others have been used to identify satellite cells in vivo and heterogeneity in the satellite cell population has been observed based on the expression of some of these markers (Kuang and Rudnicki 2008). For example, quantitative PCR analysis of single cells revealed the heterogeneity in satellite cells with respect to M-Cadherin expression (Cornelison and Wold 1997). How is this heterogeneity related, if at all, to stem cell function remains to be explored.

Pax7 marks all quiescent satellite cells and continues to mark its activated progeny until the onset of differentiation. Pax7-nGFP (transgenic as well as knock in) mice, allow efficient isolation of mouse satellite cells by FACS (Bosnakovski et al. 2008; Gayraud-Morel et al. 2012; Sambasivan et al. 2009). Satellite cells fractionated for high Pax7-nGFP (Pax7^{Hi}) manifest several stem cell features. Firstly, they express lower levels of muscle-specific genes such as Myod and Myog relative to low Pax7 (Pax7-nGFP^{Lo}) cells (Rocheteau et al 2012). Secondly, they appear to be dormant, i.e. take longer to exit mitotic quiescence when activated in culture as well as have low mitochondrial activity. Rare expression of Pax3 in adult satellite cells also identifies heterogeneity in the population within a muscle and across different muscle groups (Montarras et al. 2005).

Initial reports of heterogeneity were based on expression of *Myf5*^{nlacZ}. The *nlacZ* knock-in, encoding the β-galactosidase reporter, is expressed under the control of endogenous *Myf5* regulatory elements (Tajbakhsh et al. 1996). In the embryo, Myf5 controls the expression of muscle-specific genes and the cells expressing this factor are fated to differentiate into muscle. The majority of satellite cells express the *Myf5* reporter, while a minority of 5–10 % do not (Beauchamp et al. 2000). Apparently, this heterogeneity in contemporary expression from the *Myf5* locus could reflect the developmental heritage of satellite cell population. Employing the Cre-lox system of genetic tracing, using *Myf5*^{Cre} and *R26R*$^{stop\text{-}YFP}$ mouse lines, the embryonic progenitors expressing *Myf5* and all their descendants were permanently genetically marked (Kuang et al. 2007). This experiment revealed 5–10 % of satellite cells that never expressed *Myf5* unlike the rest. When activated to divide, *Myf5*-negative cells could generate *Myf5* expressing and nonexpressing progeny unlike the *Myf5*-positive counterparts, placing them upstream in the muscle lineage. Remarkably, in the context of transplantation, *Myf5*-negative cells were able to generate more satellite cells, i.e. self-renew more efficiently, than *Myf5*-positive population (Kuang et al. 2007). This experiment links the heterogeneity in marker expression to that in stem cell function among satellite cells. However, a similar strategy with *Myod*^{Cre} and *Mrf4*^{Cre} revealed no such heterogeneity in satellite cells with respect to history of expression of these MRFs (Kanisicak et al. 2009;

Sambasivan et al. 2013). Furthermore, *Myf5*^{Cre} crossed to a Rosa reporter mouse showed considerably less *Myf5*-negative cells after several months indicating that all satellite cells experience *Myf5* expression over time (Comai et al. in press). Though these observations appear to contradict the *Myf5*^{Cre} data, it should be noted that Cre expression only indicates the activity of corresponding MRF loci and not that of functional protein expression from these loci. Therefore, it remains plausible that the regulatory state of satellite cells and their developmental precursors with respect MRF expression could potentially influence their stem cell function.

Interestingly, a strong correlation between the heterogeneity and various types of asymmetric division in the satellite cell pool has been observed. One of the ways stem cells perform the double act of self-renewal and generation of differentiating progeny is by asymmetric cell division. Thus, asymmetric division can be linked to stem cell activity. Asymmetric partitioning of potential cell fate determining factors as well as that of template DNA strands has been reported in satellite cells (see Yennek and Tajbakhsh 2013). However, the role of such asymmetric divisions in vivo in balancing self-renewal and differentiation is yet to be elucidated.

Remarkably, satellite cells on single myofibers, enzymatically isolated and placed in culture, divide either parallel to host myofiber such that both daughters in contact with the myofiber or perpendicular to the myofiber (one daughter in contact with myofiber and another away from it). Moreover, asymmetric induction of *Myf5* occurs uniquely in the perpendicular divisions, wherein daughter contacting the myofiber acquires this factor (Kuang et al. 2007). The assumption is that if such divisions occur in vivo, the Myf5^{+} daughter proximal to the fibre will differentiate and fuse with the fibre and the Myf5^{-} daughter will replenish the satellite cell pool. Numb, an endocytic adapter shown to antagonize Notch signalling in invertebrates, is apportioned unequally to daughters of satellite cells (Conboy and Rando 2002; Shinin et al. 2006). A function of Numb in favouring self-renewal or differentiation is disputed in vertebrates as Numb does not appear to inhibit Notch activity in myogenic cells either in the embryo or adult (George et al. 2013; Jory et al. 2009; Le Roux and Tajbakhsh unpublished) and therefore, the functional outcome of this asymmetry is unclear.

Template DNA strand co-segregation is an intriguing type of asymmetric cell division. DNA strands that act as template for DNA replication (i.e. parental strands) of all chromosomes are segregated to one of the daughter cells. It has been proposed that since parental strands are free of mutations arising during replication, TDSS is a mechanism to protect stem cell genome from potentially deleterious mutations. This speculation is known as immortal strand hypothesis and remains to be tested. It is likely that differential co-segregation of parental and newly synthesized strands harbouring distinct epigenetic codes might determine the asymmetric fate outcome of the daughters (Evano and Tajbakhsh 2013; Yennek and Tajbakhsh 2013). Interestingly, a higher incidence of TDSS is observed in Pax7-nGFP^{Hi} cells (higher expression level of Pax7) 5 days post-injury of muscle underscoring distinct behaviours of these cells in the proliferation state. The functional significance of the TDSS divisions in proliferating satellite cells remains an open question. During muscle homeostasis, however, Pax7-nGFP^{Hi} and

PaxnGFP^{Lo} cells exhibit similar engraftment and long-term self-renewal capacity after serial transplantation indicating that in the quiescent state, these cells have similar potentials in spite of exhibiting diverse properties when isolated from the niche.

In summary, while satellite cells appear to be unequal on several criteria, the link between the heterogeneity by various markers is not fully understood. Importantly, a direct correlation between the heterogeneity and stem cell function of satellite cells is yet to be demonstrated in vivo.

5 Unconventional Muscle Stem Cells

Attempts at stem cell therapy for muscular dystrophies have been gaining momentum. Muscular dystrophies are a diverse group of congenital muscle wasting diseases characterised by muscle degeneration and caused by a variety of mutations. Cell therapy to regenerate the affected musculature with genetically corrected myogenic stem or progenitor cells is a much-attempted strategy. Satellite cells when isolated activate the myogenic programme and expand as myoblasts. Several attempts at transplantation failed due to poor survival of myoblasts in the host. This spurred the search for alternative stem cells with high myogenic capacity that could be genetically modified and expanded in culture and systemically delivered. A variety of cell types have been discovered to have the capacity to differentiate into muscle (Fig. 3). They range from non-satellite cells derived from muscle to neural stem cells of ectodermal origin. Studies on cell fate plasticity have also added to this list (see Tedesco et al. 2010).

When cells without muscle fate commitment fuse with the myofiber, they will come under the influence of the MRFs and could change fate to induce muscle-specific genes. Thus, fate conversion in a heterokaryon is one mechanism by which non-muscle cells could participate in myogenesis (Blau et al. 1983). Another route for myogenically uncommitted cells is to first acquire muscle progenitor identity and follow the muscle lineage progression to make differentiated muscle cells. Either way, unorthodox stem cells (non-satellite cells) apparently uncommitted to muscle fate have been shown to differentiate as muscle during regeneration. Remarkably, some of these cell types also exhibit a potential to occupy the satellite cell compartment. The capacity to make satellite cells indicates commitment occurring at progenitor level rather than accidental or controlled fusion as the mechanism. This section will describe the various non-satellite cell types reported to have myogenic capacity in the context of adult muscle regeneration (Tedesco et al. 2010) and discuss their significance vis-à-vis satellite cells in muscle stem cell function.

Bone marrow derived stem cells were one of the first non-satellite cell types shown to make muscle. When marrow-derived cells from a donor encoding muscle-specific β-gal transgene was transplanted into recipient's regenerating muscle, β-gal^{+} donor nuclei were detected in regenerated muscle fibre. Later a subset of

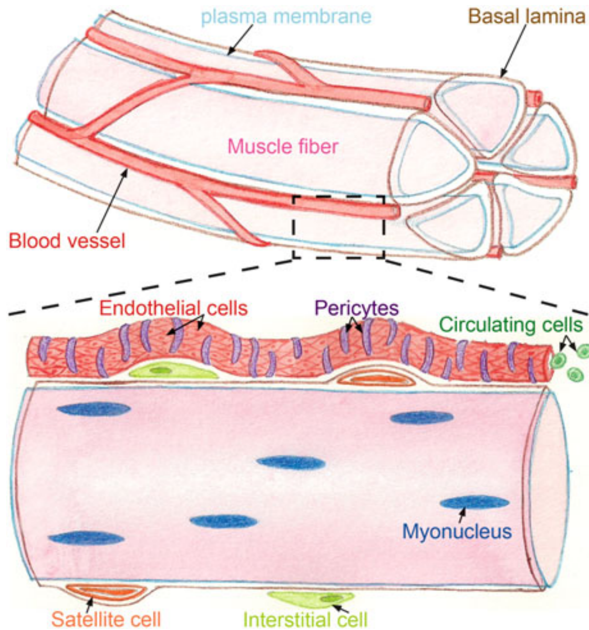


Fig. 3 The "off-beat" stem cells with muscle differentiation potential. *Top panel* shows a bundle of few fibers with associated blood vessels. In the *bottom panel*, a close-up illustration of a portion of this bundle reveals some of the non-satellite cell muscle stem cells, in relation to the muscle fibre and satellite cells. Unlike satellite cells, the interstitial cells are excluded by the basement membrane ensheathment of the muscle fibre. In particular, *Pw1* expressing interstitial cells make muscle when grafted on to regenerating muscle. Pericytes have also been shown to have myogenic capacity. Circulating stem cells, such as those with hematopoietic stem cell signature also rarely fuse into muscle when transplanted

bone marrow-derived/muscle-derived cells was shown to contribute to myofibers following transplantation. Owing to the presence of specific export channels (ABC), this subset excludes live DNA stain Hoechst and is separable as a "side population" on FACS (Ferrari et al. 1998; Gussoni et al. 1999). Muscle-derived stem cells with hematopoietic marker signature (CD45+ fraction) has similar myogenic potential. Bipotential stem cells have also been reported. Single HSC from c-Kit^{+}, Sca1^{+}, and blood lineage marker negative (Lin^{-}) subset of side population from bone marrow of CD45^{-}GFP^{+} mice reconstituted the blood of irradiated host and at the same time contributed spontaneously (without stimulated by injury) to *Panniculus carnosus* skin muscle. Such spontaneous contribution was found at a low frequency, which increased upon damage to muscle (Corbel et al. 2003). An independent study with a similar cell experimental approach showed HSC generate myeloid derivatives, which then fuse with myofibers to contribute to muscle (Camargo et al. 2003). While fusion into muscle fibre induces some muscle gene expression in grafted bone marrow-derived donor cells, they appear not to fully reprogram, as they fail to express sarcoglycan (Lapidos

et al. 2004). Though these studies have provided evidence for HSC plasticity, experiments using HSC-specific Cre recombinase driver mouse lines in combination with Cre-inducible reporter lines to trace HSC contribution to muscle during development or regeneration are needed to reveal the significance of such contribution. Nevertheless, with current evidence, the frequency of such events appears low and cannot be generalized to all muscle groups.

Mdx mice model to a certain extent human Duchenne muscular dystrophy (DMD) caused by loss of function mutations in Dystrophin, a component of membrane glycoprotein complex. Amelioration of muscle wasting could be achieved by delivering functional dystrophin into dystrophic muscle fibers. To date, the cell type found most suitable for cell therapy and has been taken to clinical trials to treat DMD, is the mesoangioblast (Sampaolesi et al. 2006; Sampaolesi et al. 2003). Blood vessel wall-associated pericytes are the source of mesangioblasts (Dellavalle et al. 2007). In fact, a Cre-loxP mouse genetic tracing study using pericyte specific TNAP-Cre (tissue non-specific alkaline phosphatase) driver has assessed the developmental plasticity of pericyte lineage towards skeletal muscle. $TNAP^+$ cells of pericyte lineage contribute to perinatal muscle growth by differentiating into muscle at low frequency and also contributing to $Pax7^+$ satellite cells (Dellavalle et al. 2011).

Other notable cell types shown to contribute to regenerating muscle upon transplantation are cells expressing a combination of endothelial (CD144 or vascular endothelial cadherin) and CD56 from human muscle (Zheng et al. 2007) as well as $PW1^+$ cells in the interstitial space between myofibers outside the basement membrane from mice (Fig. 3). $PW1^+$ cells have also been suggested to replenish the satellite cell pool (Mitchell et al. 2010). Whereas the potential of unorthodox muscle stem cells is promising and could be harnessed to treat muscular dystrophies, the discovery of the myogenic potential and especially their ability to replenish the satellite cell pool have raised a number of questions. What is the relative contribution of the various stem cells during normal muscle regeneration? What is the main mode of renewal of satellite cells? Are there muscle stem cells upstream of satellite cells in the lineage hierarchy? Four independent studies have addressed these questions by specifically ablating satellite cells. Diphtheria toxin is a bacterial protein, which selectively kills cells engineered to express active diphtheria toxin peptide (DTA) or cells expressing the human diphtheria toxin receptor (DTR). In mice carrying *Pax7-CreErt2* as well as ROSA-stop *DTA* alleles, *DTA* expression is activated in satellite cells when tamoxifen is injected (Lepper et al. 2011; McCarthy et al. 2011; Murphy et al. 2011). Intramuscular injection of DTA in $Pax7^{DTR}$ mice uniquely targets $Pax7^+$ satellite cells (Sambasivan et al. 2011). Both these strategies have been used to eliminate the majority of, or all satellite cells in adult musculature of mice. When satellite cell depleted muscle is challenged with muscle injury or strenuous exercise, it collapses owing to a dramatic failure of regeneration and restitution of myofiber regeneration occurs by transplantation of heterologous satellite cells (Sambasivan et al. 2011). These studies proved the indispensability of satellite cells to muscle regeneration and firmly established satellite cells as the major endogenous muscle stem cells.

They also indicate a possible paracrine role for satellite cells in recruiting the unorthodox stem cells to muscle lineage. Notably, freshly isolated satellite cells, not cultured in vitro, graft much more efficiently than cultured progeny of satellite cells (Ikemoto et al. 2007; Montarras et al. 2005). Recently, satellite cells cultured in Forskolin, an adenylyl cyclase activator, have been reported to retain their engraftment potential (Xu et al. 2013). This brings satellite cells back in the race as potential therapeutic cell type.

Whereas the muscle fibre is the central functional component of the tissue, skeletal muscle regeneration will be incomplete without regeneration of connective tissue. In disease states muscle function is diminished by pathological fibrosis and fat infiltration. The potential cellular source for this regeneration and pathology, fibro-adipogenic progenitors (FAPs), was recently identified using different markers; PDGFRα (Uezumi et al. 2010; Uezumi et al. 2011), CD45^{-}, CD31^{-}, Sca1^{+}, CD34^{+} (Joe et al. 2010) and Tcf4 (Murphy et al. 2011). Apparently, the FAPs identified by these various means represent the same population of progenitors. Importantly, these progenitors also expand during regenerative response and support muscle repair through paracrine influence on satellite cells (Joe et al. 2010) and are necessary for robust muscle regeneration (Murphy et al. 2011). Thus, in addition to satellite cells and unconventional stem cells with capacity/plasticity to make muscle, muscle tissue is also host to non-myogenic interstitial progenitor cell population that impacts on satellite cell behaviour.

In short, apart from satellite cells, a number of stem cell types influence or have the potential to influence muscle regeneration. In the current scenario of clinical trials for Duchenne muscular dystrophy using non-satellite stem cells and the demonstration of indispensability of satellite cells in muscle regeneration, the significance of understanding the role of satellite cells vis-à-vis other stem cells is accentuated and needs to be explored.

6 Ageing and Regenerative Capacity of Satellite Cells

Ageing is characterized by decline in homeostatic maintenance of tissue structure and function as well as blunted regenerative response. Affected stem cell function is partially responsible for this decline. Sarcopenia, age-related muscle loss, is a major health concern. The impact of ageing on satellite cell function has been studied extensively. The effect on satellite cell function is due to changes in the milieu [reviewed in Hikida (2011)], local and systemic, as well as cell intrinsic changes. The result is poor activation/proliferation and also defective differentiation.

Although, depletion of satellite cells with age has been reported, there is controversy in the field on age-related reduction of satellite cell number (Garcia-Prat et al. 2013; Tajbakhsh 2013). This is likely due to differences in the experimental approaches such as markers used for enumeration as well as age of the animals used for the study. Poor self-renewal capacity owing to changes in cues from niche affects maintenance of satellite cell number. Increased FGF2 in aged

muscle downregulates Sprouty 1, a self-renewal promoting factor (Shea et al. 2010), and thus compromises satellite cell quiescence leading to a decline in the number of satellite cells (Chakkalakal et al. 2012).

Increased levels of TGFβ in aged muscle causes reduced regenerative potential of satellite cells. The effector of TGFβ, phospho-Smad3, induces cell cycle inhibitors, an action countered by Notch signalling, to limit satellite cell proliferation (Carlson et al. 2008). Notch signalling is also directly affected in aged muscle. The failure to induce Delta1 in ageing muscle fibers could be one of the reasons for reduced activation of satellite cells and therefore, poor regenerative response of aged muscle (Conboy et al. 2003). Similarly, systemic increase in Wnt has been implicated in defective differentiation of satellite cells, i.e. myogenic to fibrogenic switch (Brack et al. 2007). The role of "old", local, as well as systemic, milieu in blunting satellite cell function has been demonstrated by heterochronic transplants and heterochronic parabiosis experiments. Transplantation into young muscle bed supports efficient regeneration of old donor-derived satellite cell (Garcia-Prat et al. 2013). In parabionts, wherein the circulation of two mice an old and another young are joined, the regenerative potential of the old satellite cells is dramatically improved (Conboy et al. 2005). Altered inflammatory response, local and systemic, upon ageing could also impact satellite cells (Shavlakadze et al. 2010). These key experiments showed that the intrinsic changes in the satellite cells notwithstanding, amelioration of cues in the local and systemic environment could rejuvenate satellite cell response to regenerative demand. These findings have raised therapeutic hopes for patients suffering from sarcopenia.

Concluding Remarks

Since their discovery in 1960s, satellite cells have been studied extensively. Propelled by their possible therapeutic potential for treatment of muscle degenerative diseases such as dystrophies and sarcopenia, satellite cell properties have been investigated in detail. Consequently, they have emerged as an excellent model to understand adult tissue-specific stem cell biology. The hunt for cells better suited for stem cell therapy of muscle wasting diseases has revealed the plasticity of several non-satellite cells to make skeletal muscle. However, the recent findings of the indispensability of satellite cells for muscle regeneration and of inability of other stem cells to replenish the experimentally depleted satellite cell pool, firmly establishes satellite cells as the major adult stem cell compartment of the skeletal muscle tissue. The failure of satellite cell-derived myogenic cells to transplant due to limited migratory capacity in the muscle bed had deterred their application in regenerative medicine. However, the successful transplantation of freshly isolated, non-cultured satellite cells has underlined the need to further understand the mechanisms controlling satellite cell "stemness" and activation. These studies would help to turn the therapeutic dream into a reality.

Acknowledgements RS is supported by Ramalingaswamy Fellowship from Department of Biotechnology, Government of India as well as by Institute for Stem Cell Biology and Regenerative Medicine (inStem). ST acknowledges support from the Institut Pasteur, Centre National de la Recherche Scientifique, Association Française contre les Myopathies (AFM), and the Fondation pour la Recherche Médicale. We are thankful to the artist Mr. Sartaj Ghuman for the illustrations.

References

Abou-Khalil R, Le Grand F, Pallafacchina G, Valable S, Authier FJ, Rudnicki MA, Gherardi RK, Germain S, Chretien F, Sotiropoulos A, Lafuste P, Montarras D, Chazaud B (2009) Autocrine and paracrine angiopoietin 1/Tie-2 signaling promotes muscle satellite cell self-renewal. Cell Stem Cell 5:298–309

Armand O, Boutineau AM, Mauger A, Pautou MP, Kieny M (1983) Origin of satellite cells in avian skeletal muscles. Arch Anat Microsc Morphol Exp 72:163–81

Asakura A, Hirai H, Kablar B, Morita S, Ishibashi J, Piras BA, Christ AJ, Verma M, Vineretsky KA, Rudnicki MA (2007) Increased survival of muscle stem cells lacking the MyoD gene after transplantation into regenerating skeletal muscle. Proc Natl Acad Sci U S A 104:16552–7

Beauchamp JR, Heslop L, Yu DS, Tajbakhsh S, Kelly RG, Wernig A, Buckingham ME, Partridge TA, Zammit PS (2000) Expression of CD34 and Myf5 defines the majority of quiescent adult skeletal muscle satellite cells. J Cell Biol 151:1221–34

Ben-Yair R, Kalcheim C (2005) Lineage analysis of the avian dermomyotome sheet reveals the existence of single cells with both dermal and muscle progenitor fates. Development 132:689–701

Bischoff R (1975) Regeneration of single skeletal muscle fibers in vitro. Anat Rec 182:215–35

Bischoff R (1990) Interaction between satellite cells and skeletal muscle fibers. Development 109:943–52

Bjornson CR, Cheung TH, Liu L, Tripathi PV, Steeper KM, Rando TA (2012) Notch signaling is necessary to maintain quiescence in adult muscle stem cells. Stem Cells 30:232–42

Blau HM, Chiu CP, Webster C (1983) Cytoplasmic activation of human nuclear genes in stable heterocaryons. Cell 32:1171–80

Bosnakovski D, Xu Z, Li W, Thet S, Cleaver O, Perlingeiro RC, Kyba M (2008) Prospective isolation of skeletal muscle stem cells with a Pax7 reporter. Stem Cells 26:3194–204

Brack AS, Conboy MJ, Roy S, Lee M, Kuo CJ, Keller C, Rando TA (2007) Increased Wnt signaling during aging alters muscle stem cell fate and increases fibrosis. Science 317:807–10

Brack AS, Conboy IM, Conboy MJ, Shen J, Rando TA (2008) A temporal switch from notch to Wnt signaling in muscle stem cells is necessary for normal adult myogenesis. Cell Stem Cell 2:50–9

Brohl D, Vasyutina E, Czajkowski MT, Griger J, Rassek C, Rahn HP, Purfurst B, Wende H, Birchmeier C (2012) Colonization of the satellite cell niche by skeletal muscle progenitor cells depends on Notch signals. Dev Cell 23:469–81

Budry L, Balsalobre A, Gauthier Y, Khetchoumian K, L'Honore A, Vallette S, Brue T, Figarella-Branger D, Meij B, Drouin J (2012) The selector gene Pax7 dictates alternate pituitary cell fates through its pioneer action on chromatin remodeling. Genes Dev 26:2299–310

Camargo FD, Green R, Capetanaki Y, Jackson KA, Goodell MA (2003) Single hematopoietic stem cells generate skeletal muscle through myeloid intermediates. Nat Med 9:1520–7

Carlson ME, Hsu M, Conboy IM (2008) Imbalance between pSmad3 and Notch induces CDK inhibitors in old muscle stem cells. Nature 454:528–32

Chakkalakal JV, Jones KM, Basson MA, Brack AS (2012) The aged niche disrupts muscle stem cell quiescence. Nature 490:355–60

Collins CA, Olsen I, Zammit PS, Heslop L, Petrie A, Partridge TA, Morgan JE (2005) Stem cell function, self-renewal, and behavioral heterogeneity of cells from the adult muscle satellite cell niche. Cell 122:289–301

Comai G, Sambasivan R, Gopalakrishnan S, Tajbakhsh S (2014) Variations in the efficiency of lineage marking and ablation confound distinctions between myogenic cell populations. Dev Cell (in press)

Conboy IM, Rando TA (2002) The regulation of Notch signaling controls satellite cell activation and cell fate determination in postnatal myogenesis. Dev Cell 3:397–409

Conboy IM, Conboy MJ, Smythe GM, Rando TA (2003) Notch-mediated restoration of regenerative potential to aged muscle. Science 302:1575–7

Conboy IM, Conboy MJ, Wagers AJ, Girma ER, Weissman IL, Rando TA (2005) Rejuvenation of aged progenitor cells by exposure to a young systemic environment. Nature 433:760–4

Corbel SY, Lee A, Yi L, Duenas J, Brazelton TR, Blau HM, Rossi FM (2003) Contribution of hematopoietic stem cells to skeletal muscle. Nat Med 9:1528–32

Cornelison DDW, Wold BJ (1997) Single-cell analysis of regulatory gene expression in quiescent and activated mouse skeletal muscle satellite cells. Dev Biol 191:270–283

Cornelison DD, Olwin BB, Rudnicki MA, Wold BJ (2000) MyoD(-/-) satellite cells in single-fiber culture are differentiation defective and MRF4 deficient. Dev Biol 224:122–37

Day RM, Cioce V, Breckenridge D, Castagnino P, Bottaro DP (1999) Differential signaling by alternative HGF isoforms through c-Met: activation of both MAP kinase and PI 3-kinase pathways is insufficient for mitogenesis. Oncogene 18:3399–406

Dellavalle A, Sampaolesi M, Tonlorenzi R, Tagliafico E, Sacchetti B, Perani L, Innocenzi A, Galvez BG, Messina G, Morosetti R, Li S, Belicchi M, Peretti G, Chamberlain JS, Wright WE, Torrente Y, Ferrari S, Bianco P, Cossu G (2007) Pericytes of human skeletal muscle are myogenic precursors distinct from satellite cells. Nat Cell Biol 9:255–67

Dellavalle A, Maroli G, Covarello D, Azzoni E, Innocenzi A, Perani L, Antonini S, Sambasivan R, Brunelli S, Tajbakhsh S, Cossu G (2011) Pericytes resident in postnatal skeletal muscle differentiate into muscle fibers and generate satellite cells. Nat Commun 2:499

Evano B, Tajbakhsh S (2013) Sorting DNA with asymmetry: a new player in gene regulation? Chromosome Res 21:225–42

Ferrari G, Cusella-De Angelis G, Coletta M, Paolucci E, Stornaiuolo A, Cossu G, Mavilio F (1998) Muscle regeneration by bone marrow-derived myogenic progenitors. Science 279:1528–30 [see comments] [published erratum appears in Science 1998 Aug 14;281(5379):923]

Floss T, Arnold HH, Braun T (1997) A role for FGF-6 in skeletal muscle regeneration. Genes Dev 11:2040–51

Fukada SI, Uezumi A, Ikemoto M, Masuda S, Segawa M, Tanimura N, Yamamoto H, Miyagoe-Suzuki Y, Takeda S (2007) Molecular signature of quiescent satellite cells In adult skeletal muscle. Stem Cells 25:2448–59

Fukada S, Yamaguchi M, Kokubo H, Ogawa R, Uezumi A, Yoneda T, Matev MM, Motohashi N, Ito T, Zolkiewska A, Johnson RL, Saga Y, Miyagoe-Suzuki Y, Tsujikawa K, Takeda S, Yamamoto H (2011) Hesr1 and Hesr3 are essential to generate undifferentiated quiescent satellite cells and to maintain satellite cell numbers. Development 138:4609–19

Garcia-Prat L, Sousa-Victor P, Munoz-Canoves P (2013) Functional dysregulation of stem cells during aging: a focus on skeletal muscle stem cells. FEBS J 280:4051–62

Gayraud-Morel B, Chretien F, Tajbakhsh S (2009) Skeletal muscle as a paradigm for regenerative biology and medicine. Regen Med 4:293–319

Gayraud-Morel B, Chretien F, Jory A, Sambasivan R, Negroni E, Flamant P, Soubigou G, Coppee JY, Di Santo J, Cumano A, Mouly V, Tajbakhsh S (2012) Myf5 haploinsufficiency reveals distinct cell fate potentials for adult skeletal muscle stem cells. J Cell Sci 125:1738–49

George RM, Biressi S, Beres BJ, Rogers E, Mulia AK, Allen RE, Rawls A, Rando TA, Wilson-Rawls J (2013) Numb-deficient satellite cells have regeneration and proliferation defects. Proc Natl Acad Sci USA 110:18549–18554

Goulding MD, Chalepakis G, Deutsch U, Erselius JR, Gruss P (1991) Pax-3, a novel murine DNA binding protein expressed during early neurogenesis. EMBO J 10:1135–47

Gros J, Manceau M, Thome V, Marcelle C (2005) A common somitic origin for embryonic muscle progenitors and satellite cells. Nature 435:954–8

Gunther S, Kim J, Kostin S, Lepper C, Fan CM, Braun T (2013) Myf5-positive satellite cells contribute to Pax7-dependent long-term maintenance of adult muscle stem cells. Cell Stem Cell 13:590–601

Gussoni E, Soneoka Y, Strickland CD, Buzney EA, Khan MK, Flint AF, Kunkel LM, Mulligan RC (1999) Dystrophin expression in the mdx mouse restored by stem cell transplantation. Nature 401:390–394

Halevy O, Cantley LC (2004) Differential regulation of the phosphoinositide 3-kinase and MAP kinase pathways by hepatocyte growth factor vs. insulin-like growth factor-I in myogenic cells. Exp Cell Res 297:224–34

Harel I, Nathan E, Tirosh-Finkel L, Zigdon H, Guimaraes-Camboa N, Evans SM, Tzahor E (2009) Distinct origins and genetic programs of head muscle satellite cells. Dev Cell 16:822–32

Hasty P, Bradley A, Morris JH, Edmondson DG, Venuti JM, Olson EN, Klein WH (1993) Muscle deficiency and neonatal death in mice with a targeted mutation in the myogenin gene. Nature 364:501–506

Hikida RS (2011) Aging changes in satellite cells and their functions. Curr Aging Sci 4:279–97

Horst D, Ustanina S, Sergi C, Mikuz G, Juergens H, Braun T, Vorobyov E (2006) Comparative expression analysis of Pax3 and Pax7 during mouse myogenesis. Int J Dev Biol 50:47–54

Ikemoto M, Fukada S, Uezumi A, Masuda S, Miyoshi H, Yamamoto H, Wada MR, Masubuchi N, Miyagoe-Suzuki Y, Takeda S (2007) Autologous transplantation of SM/C-2.6(+) satellite cells transduced with micro-dystrophin CS1 cDNA by lentiviral vector into mdx mice. Mol Ther 15:2178–85

Illa I, Leon-Monzon M, Dalakas MC (1992) Regenerating and denervated human muscle fibers and satellite cells express neural cell adhesion molecule recognized by monoclonal antibodies to natural killer cells. Ann Neurol 31:46–52

Joe AW, Yi L, Natarajan A, Le Grand F, So L, Wang J, Rudnicki MA, Rossi FM (2010) Muscle injury activates resident fibro/adipogenic progenitors that facilitate myogenesis. Nat Cell Biol 12:153–63

Jones NC, Fedorov YV, Rosenthal RS, Olwin BB (2001) ERK1/2 is required for myoblast proliferation but is dispensable for muscle gene expression and cell fusion. J Cell Physiol 186:104–15

Jory A, Le Roux I, Gayraud-Morel B, Rocheteau P, Cohen-Tannoudji M, Cumano A, Tajbakhsh S (2009) Numb promotes an increase in skeletal muscle progenitor cells in the embryonic somite. Stem Cells 27:2769–80

Kanisicak O, Mendez JJ, Yamamoto S, Yamamoto M, Goldhamer DJ (2009) Progenitors of skeletal muscle satellite cells express the muscle determination gene, MyoD. Dev Biol 332:131–41

Kassar-Duchossoy L, Giacone E, Gayraud-Morel B, Jory A, Gomes D, Tajbakhsh S (2005) Pax3/Pax7 mark a novel population of primitive myogenic cells during development. Genes Dev 19:1426–31

Katz B (1961) The terminations of the afferent nerve fiber in the muscle spindle of the frog. Philos Trans R Soc Lond B Biol Sci 243:221–240

Kharraz Y, Guerra J, Mann CJ, Serrano AL, Munoz-Canoves P (2010) Macrophage plasticity and the role of inflammation in skeletal muscle repair. Mediators Inflamm 2013:491497

Kitamoto T, Hanaoka K (2010) Notch3 null mutation in mice causes muscle hyperplasia by repetitive muscle regeneration. Stem Cells 28:2205–16

Kitzmann M, Carnac G, Vandromme M, Primig M, Lamb NJC, Fernandez A (1998) The muscle regulatory factors Myod and Myf-5 undergo distinct cell cycle-specific expression in muscle cells. J Cell Biol 142:1447–1459

Konigsberg UR, Lipton BH, Konigsberg IR (1975) The regenerative response of single mature muscle fibers isolated in vitro. Dev Biol 45:260–75

Kuang S, Rudnicki MA (2008) The emerging biology of satellite cells and their therapeutic potential. Trends Mol Med 14:82–91

Kuang S, Charge SB, Seale P, Huh M, Rudnicki MA (2006) Distinct roles for Pax7 and Pax3 in adult regenerative myogenesis. J Cell Biol 172:103–13

Kuang S, Kuroda K, Le Grand F, Rudnicki MA (2007) Asymmetric self-renewal and commitment of satellite stem cells in muscle. Cell 129:999–1010

Lapidos KA, Chen YE, Earley JU, Heydemann A, Huber JM, Chien M, Ma A, McNally EM (2004) Transplanted hematopoietic stem cells demonstrate impaired sarcoglycan expression after engraftment into cardiac and skeletal muscle. J Clin Invest 114:1577–85

Le Grand F, Jones AE, Seale V, Scime A, Rudnicki MA (2009) Wnt7a activates the planar cell polarity pathway to drive the symmetric expansion of satellite stem cells. Cell Stem Cell 4:535–47

Lee SJ, Huynh TV, Lee YS, Sebald SM, Wilcox-Adelman SA, Iwamori N, Lepper C, Matzuk MM, Fan CM (2012) Role of satellite cells versus myofibers in muscle hypertrophy induced by inhibition of the myostatin/activin signaling pathway. Proc Natl Acad Sci U S A 109:E2353–60

Lepper C, Conway SJ, Fan CM (2009) Adult satellite cells and embryonic muscle progenitors have distinct genetic requirements. Nature 460:627–31

Lepper C, Partridge TA, Fan CM (2011) An absolute requirement for Pax7-positive satellite cells in acute injury-induced skeletal muscle regeneration. Development 138:3639–46

Mansouri A, Stoykova A, Torres M, Gruss P (1996) Dysgenesis of cephalic neural crest derivatives in Pax7-/- mutant mice. Development 122:831–8

Mauro A (1961) Satellite cell of skeletal muscle fibers. J Biophys Biochem Cytol 9:493–5

McCarthy JJ, Mula J, Miyazaki M, Erfani R, Garrison K, Farooqui AB, Srikuea R, Lawson BA, Grimes B, Keller C, Van Zant G, Campbell KS, Esser KA, Dupont-Versteegden EE, Peterson CA (2011) Effective fiber hypertrophy in satellite cell-depleted skeletal muscle. Development 138:3657–66

McKinnell IW, Ishibashi J, Le Grand F, Punch VG, Addicks GC, Greenblatt JF, Dilworth FJ, Rudnicki MA (2008) Pax7 activates myogenic genes by recruitment of a histone methyltransferase complex. Nat Cell Biol 10:77–84

Meadows E, Cho JH, Flynn JM, Klein WH (2008) Myogenin regulates a distinct genetic program in adult muscle stem cells. Dev Biol 322:406–14

Meadows E, Flynn JM, Klein WH (2011) Myogenin regulates exercise capacity but is dispensable for skeletal muscle regeneration in adult mdx mice. PLoS One 6:e16184

Megeney LA, Kablar B, Garrett K, Anderson JE, Rudnicki MA (1996) MyoD is required for myogenic stem cell function in adult skeletal muscle. Genes Dev 10:1173–1183

Mitchell JK, Pannerec A, Cadot B, Parlakian A, Besson V, Gomes RE, Marazzi G, Sassoon AD (2010) Identification and characterization of a non-satellite cell muscle resident progenitor during postnatal development. Nat Cell Biol 12:257–266

Moncaut N, Rigby PW, Carvajal JJ (2013) Dial M(RF) for myogenesis. FEBS J 280:3980–90

Montarras D, Morgan J, Collins C, Relaix F, Zaffran S, Cumano A, Partridge T, Buckingham M (2005) Direct isolation of satellite cells for skeletal muscle regeneration. Science 309:2064–7

Mourikis P, Tajbakhsh S (2014) Distinct contextual roles for Notch signalling in skeletal muscle stem cells. BMC Dev Biol 14:2

Mourikis P, Gopalakrishnan S, Sambasivan R, Tajbakhsh S (2012a) Cell-autonomous Notch activity maintains the temporal specification potential of skeletal muscle stem cells. Development 139:4536–48

Mourikis P, Sambasivan R, Castel D, Rocheteau P, Bizzarro V, Tajbakhsh S (2012b) A critical requirement for notch signaling in maintenance of the quiescent skeletal muscle stem cell state. Stem Cells 30:243–52

Murphy M, Kardon G (2011) Origin of vertebrate limb muscle: The role of progenitor and myoblast populations. Curr Top Dev Biol 96:1–32

Murphy MM, Lawson JA, Mathew SJ, Hutcheson DA, Kardon G (2011) Satellite cells, connective tissue fibroblasts and their interactions are crucial for muscle regeneration. Development 138:3625–37

Murphy MM, Keefe CA, Lawson JA, Flygare DS, Yandell M, Kardon G (2014) Transiently active Wnt/β-catenin signaling is not required but must be silenced for stem cell function during muscle regeneration. Stem Cell Reports 3:1–14

Nabeshima Y, Hanaoka K, Hayasaka M, Esumi E, Li S, Nonaka I, Nabeshima Y (1993) Myogenin gene disruption results in perinatal lethality because of severe muscle defect. Nature 364:532–535

Nagata Y, Partridge TA, Matsuda R, Zammit PS (2006) Entry of muscle satellite cells into the cell cycle requires sphingolipid signaling. J Cell Biol 174:245–53

Otto A, Schmidt C, Luke G, Allen S, Valasek P, Muntoni F, Lawrence-Watt D, Patel K (2008) Canonical Wnt signalling induces satellite-cell proliferation during adult skeletal muscle regeneration. J Cell Sci 121:2939–50

Oustanina S, Hause G, Braun T (2004) Pax7 directs postnatal renewal and propagation of myogenic satellite cells but not their specification. EMBO J 23:3430–9

Perry JM, Li L (2010) Functional assays for hematopoietic stem cell self-renewal. Methods Mol Biol 636:45–54

Relaix F, Rocancourt D, Mansouri A, Buckingham M (2005) A Pax3/Pax7-dependent population of skeletal muscle progenitor cells. Nature 435:948–53

Relaix F, Montarras D, Zaffran S, Gayraud-Morel B, Rocancourt D, Tajbakhsh S, Mansouri A, Cumano A, Buckingham M (2006) Pax3 and Pax7 have distinct and overlapping functions in adult muscle progenitor cells. J Cell Biol 172:91–102

Rocheteau P, Gayraud-Morel B, Siegl-Cachedenier I, Blasco MA, Tajbakhsh S (2012) A subpopulation of adult skeletal muscle stem cells retains all template DNA strands after cell division. Cell 148:112–25

Sacco A, Doyonnas R, Kraft P, Vitorovic S, Blau HM (2008) Self-renewal and expansion of single transplanted muscle stem cells. Nature 456:502–6

Sambasivan R, Gayraud-Morel B, Dumas G, Cimper C, Paisant S, Kelly RG, Tajbakhsh S (2009) Distinct regulatory cascades govern extraocular and pharyngeal arch muscle progenitor cell fates. Dev Cell 16:810–21

Sambasivan R, Yao R, Kissenpfennig A, Van Wittenberghe L, Paldi A, Gayraud-Morel B, Guenou H, Malissen B, Tajbakhsh S, Galy A (2011) Pax7-expressing satellite cells are indispensable for adult skeletal muscle regeneration. Development 138:3647–56

Sambasivan R, Comai G, Le Roux I, Gomes D, Konge J, Dumas G, Cimper C, Tajbakhsh S (2013) Embryonic founders of adult muscle stem cells are primed by the determination gene Mrf4. Dev Biol 381:241–55

Sampaolesi M, Torrente Y, Innocenzi A, Tonlorenzi R, D'Antona G, Pellegrino MA, Barresi R, Bresolin N, De Angelis MG, Campbell KP, Bottinelli R, Cossu G (2003) Cell therapy of alpha-sarcoglycan null dystrophic mice through intra-arterial delivery of mesoangioblasts. Science 301:487–92

Sampaolesi M, Blot S, D'Antona G, Granger N, Tonlorenzi R, Innocenzi A, Mognol P, Thibaud JL, Galvez BG, Barthelemy I, Perani L, Mantero S, Guttinger M, Pansarasa O, Rinaldi C, Cusella De Angelis MG, Torrente Y, Bordignon C, Bottinelli R, Cossu G (2006) Mesoangioblast stem cells ameliorate muscle function in dystrophic dogs. Nature 444:574–9

Scharner J, Zammit PS (2011) The muscle satellite cell at 50: the formative years. Skelet Muscle 1:28

Schienda J, Engleka KA, Jun S, Hansen MS, Epstein JA, Tabin CJ, Kunkel LM, Kardon G (2006) Somitic origin of limb muscle satellite and side population cells. Proc Natl Acad Sci U S A 103:945–50

Schuster-Gossler K, Cordes R, Gossler A (2007) Premature myogenic differentiation and depletion of progenitor cells cause severe muscle hypotrophy in Delta1 mutants. Proc Natl Acad Sci U S A 104:537–42

Seale P, Sabourin LA, Girgis-Gabardo A, Mansouri A, Gruss P, Rudnicki MA (2000) Pax7 is required for the specification of myogenic satellite cells. Cell 102:777–86
Shavlakadze T, McGeachie J, Grounds MD (2010) Delayed but excellent myogenic stem cell response of regenerating geriatric skeletal muscles in mice. Biogerontology 11:363–76
Shea KL, Xiang W, LaPorta VS, Licht JD, Keller C, Basson MA, Brack AS (2010) Sprouty1 regulates reversible quiescence of a self-renewing adult muscle stem cell pool during regeneration. Cell Stem Cell 6:117–29
Shinin V, Gayraud-Morel B, Gomes D, Tajbakhsh S (2006) Asymmetric division and cosegregation of template DNA strands in adult muscle satellite cells. Nat Cell Biol 8:677–82
Snow MH (1977) Myogenic cell formation in regenerating rat skeletal muscle injured by mincing. II An autoradiographic study. Anat Rec 188:201–17
Soleimani VD, Punch VG, Kawabe Y, Jones AE, Palidwor GA, Porter CJ, Cross JW, Carvajal JJ, Kockx CE, van IJcken WF, Perkins TJ, Rigby PW, Grosveld F, Rudnicki MA (2012) Transcriptional dominance of Pax7 in adult myogenesis is due to high-affinity recognition of homeodomain motifs. Dev Cell 22:1208–20
Spalding KL, Bhardwaj RD, Buchholz BA, Druid H, Frisen J (2005) Retrospective birth dating of cells in humans. Cell 122:133–43
Subramaniam S, Sreenivas P, Cheedipudi S, Reddy VR, Shashidhara LS, Chilukoti RK, Mylavarapu M, Dhawan J (2013) Distinct transcriptional networks in quiescent myoblasts: a role for Wnt signaling in reversible vs irreversible arrest. PLoS One 8:e65097
Tajbakhsh S (2009) Skeletal muscle stem cells in developmental versus regenerative myogenesis. J Intern Med 266:372–389
Tajbakhsh S (2013) Losing stem cells in the aged skeletal muscle niche. Cell Res 23:455–7
Tajbakhsh S, Rocancourt D, Buckingham M (1996) Muscle progenitor cells failing to respond to positional cues adopt non-myogenic fates in myf-5 null mice. Nature 384:266–270
Tajbakhsh S, Rocancourt D, Cossu G, Buckingham M (1997) Redefining the genetic hierarchies controlling skeletal myogenesis: Pax-3 and Myf-5 act upstream of MyoD. Cell 89:127–138
Tatsumi R, Anderson JE, Nevoret CJ, Halevy O, Allen RE (1998) HGF/SF is present in normal adult skeletal muscle and is capable of activating satellite cells. Dev Biol 194:114–28
Tedesco FS, Dellavalle A, Diaz-Manera J, Messina G, Cossu G (2010) Repairing skeletal muscle: regenerative potential of skeletal muscle stem cells. J Clin Invest 120:11–9
Uezumi A, Fukada S, Yamamoto N, Takeda S, Tsuchida K (2010) Mesenchymal progenitors distinct from satellite cells contribute to ectopic fat cell formation in skeletal muscle. Nat Cell Biol 12:143–52
Uezumi A, Ito T, Morikawa D, Shimizu N, Yoneda T, Segawa M, Yamaguchi M, Ogawa R, Matev MM, Miyagoe-Suzuki Y, Takeda S, Tsujikawa K, Tsuchida K, Yamamoto H, Fukada S (2011) Fibrosis and adipogenesis originate from a common mesenchymal progenitor in skeletal muscle. J Cell Sci 124:3654–64
Ustanina S, Carvajal J, Rigby P, Braun T (2007) The myogenic factor Myf5 supports efficient skeletal muscle regeneration by enabling transient myoblast amplification. Stem Cells 25:2006–16
Vinciguerra M, Musaro A, Rosenthal N (2010) Regulation of muscle atrophy in aging and disease. Adv Exp Med Biol 694:211–33
von Maltzahn J, Jones AE, Parks RJ, Rudnicki MA (2013) Pax7 is critical for the normal function of satellite cells in adult skeletal muscle. Proc Natl Acad Sci U S A 110:16474–9
Wen Y, Bi P, Liu W, Asakura A, Keller C, Kuang S (2012) Constitutive Notch activation upregulates Pax7 and promotes the self-renewal of skeletal muscle satellite cells. Mol Cell Biol 32:2300–11
White JD, Scaffidi A, Davies M, McGeachie J, Rudnicki MA, Grounds MD (2000) Myotube formation is delayed but not prevented in MyoD-deficient skeletal muscle: studies in regenerating whole muscle grafts of adult mice. J Histochem Cytochem 48:1531–44
White RB, Bierinx AS, Gnocchi VF, Zammit PS (2010) Dynamics of muscle fiber growth during postnatal mouse development. BMC Dev Biol 10:21

Xu C, Tabebordbar M, Iovino S, Ciarlo C, Liu J, Castiglioni A, Price E, Liu M, Barton ER, Kahn CR, Wagers AJ, Zon LI (2013) A Zebrafish embryo culture system defines factors that promote vertebrate myogenesis across species. Cell 155:909–21

Yablonka-Reuveni Z, Rudnicki MA, Rivera AJ, Primig M, Anderson JE, Natanson P (1999) The transition from proliferation to differentiation is delayed in satellite cells from mice lacking MyoD. Dev Biol 210:440–55

Yennek S, Tajbakhsh S (2013) DNA asymmetry and cell fate regulation in stem cells. Semin Cell Dev Biol 24:627–42

Yin H, Price F, Rudnicki MA (2013) Satellite cells and the muscle stem cell niche. Physiol Rev 93:23–67

Zanou N, Gailly P (2013) Skeletal muscle hypertrophy and regeneration: interplay between the myogenic regulatory factors (MRFs) and insulin-like growth factors (IGFs) pathways. Cell Mol Life Sci 70:4117–30

Zheng B, Cao B, Crisan M, Sun B, Li G, Logar A, Yap S, Pollett JB, Drowley L, Cassino T, Gharaibeh B, Deasy BM, Huard J, Peault B (2007) Prospective identification of myogenic endothelial cells in human skeletal muscle. Nat Biotechnol 25:1025–34

Dormancy and Quiescence of Skeletal Muscle Stem Cells

Pierre Rocheteau, Mathilde Vinet, and Fabrice Chretien

Abstract The skeletal muscle of vertebrates has a huge regenerative capacity. When destroyed after different types of injury, this organ can regenerate very quickly (less than 20 days following myotoxin injection in the mouse) *ad integrum* and repeatedly. The cell responsible for this regeneration is the so-called satellite cell, the muscle stem cell that lies on top of the muscle fibre, a giant, multinucleated cell that contains the contractile material. When injected in the muscle, satellite cells can efficiently differentiate into contractile muscle fibres. The satellite cell shows great therapeutic potential; and its regenerative capacity has triggered particular interest in the field of muscular degeneration.

In this review we will focus on one particular property of the satellite cell: its quiescence and dormancy. Indeed adult satellite cells are quiescent; they lie between the basal lamina and the basement membrane of the muscle fibre, ready to proliferate, and fuse in order to regenerate myofibers upon injury. It has recently been shown that a subpopulation of satellite cells is able to enter dormancy in human and mice cadavers. Dormancy is defined by a low metabolic state, low mobility, and a long lag before division when plated in vitro, compared to quiescent cells. This definition is also based on current knowledge about long-term hematopoietic stem cells, a subpopulation of stem cells that are described as dormant based on the same criteria (rare division and low metabolism when compared to progeny which are dividing more often).

In the first part of this review, we will provide a description of satellite cells which addresses their quiescent state. We will then focus on the uneven distribution of satellite cells in the muscle and describe evidence that suggests that their dormancy differs from one muscle to the next and that one should be cautious when making generalisations regarding this cellular state.

In a second part, we will discuss the transition between active dividing cells in developing animals to quiescence. This mechanism could be used or amplified in the switch from quiescence to dormancy.

P. Rocheteau • M. Vinet • F. Chretien (✉)
Human histopathology and animal models, Institut Pasteur, 28 rue du Dr. Roux, 75724 Paris Cedex 15, France
e-mail: fabrice.chretien@pasteur.fr

© Springer-Verlag Berlin Heidelberg 2015

B. Brand-Saberi (ed.), *Vertebrate Myogenesis*, Results and Problems in Cell Differentiation 56, DOI 10.1007/978-3-662-44608-9_10

In a third part, we will review the signals and dynamics that actively maintain the satellite cell quiescent. The in-depth understanding of these mechanisms is key to describing how dormancy relies on quiescent state of the cells.

In a fourth part, we will deal with dormancy per se: how dormant satellite cells can be obtained, their characteristics, their metabolic profile, and their molecular signature as compared to quiescent cells. Here, we will highlight one of the most important recent findings: that quiescence is a prerequisite for the entry of the satellite cell into dormancy.

Since dormancy is a newly discovered phenomenon, we will review the mechanisms responsible for quiescence and activation, as these two cellular states are better known and key to understanding satellite cell dormancy. This will allow us to describe dormancy and its prerequisites.

1 The Satellite Cell, the Discovery of the Quiescent Muscle Stem Cell

1.1 The First Electron Microscopy Observations Hint to the Quiescent State of the Satellite Cell

Satellite cells were first described in 1961 by Mauro, following electron microscopy observations of adult frog muscles. This seminal observation contains critical information regarding the anatomy of the cells as "wedged between the plasma membrane of the muscle fiber and the basement membrane [. . .] the surface of the fiber is not distorted outward, but instead the satellite cell protrudes inward pushing the myofibrils of the muscle cell aside" (Mauro 1961). Complementary observation by electron microscopy allowed the description of this cell-type as apparently quiescent, due to the state of their chromatin (Mauro 1961).

Satellite cells are spindle-shaped with a central nucleus located close to the outer surface of the muscle fibres. They are located in a depression or in a groove on the fibre and are covered by a common and continuous basal lamina. The gap between the satellite and fibre is about 15 nm and this close apposition is maintained throughout the area of cell contact (Guth and Yellin 1971; Guth 1973).

Satellite cells have an oval-shaped nucleus, often in line with adjacent myonuclei. This nucleus contains more heterochromatin than do the myonuclei, which suggests a less active transcriptional state and constitutes another argument in favour of a less active or quiescent state.

1.2 The Distribution of Satellite Cells on the Different Muscle Fibres Could be Testify to Differential Activity

Satellite cells exist in all vertebrate species, but their number per muscle varies widely in the adult depending on species, age, muscle location, and muscle type. While satellite cells represent 3–10 % of the sub-basal lamina population of muscle nuclei (de Maruenda and Franzini-Armstrong 1978), their proportion along the myofiber at the neuromuscular junctions in rat (Kelly 1978) and human (Wokke et al. 1989) muscle is 20-fold greater. In addition, although no functional association has been found between satellite cells and the neuromuscular junction, innervation has been reported to influence the proliferation of associated satellite cells.

The number of satellite cells varies with fibre type. Slow muscle fibres contain three to four times more satellite cells than do fast muscle fibres (Kelly 1978; Gibson and Schultz 1982). For example, there are 5,000 satellite cells per cubic millimetre in the rat soleus (slow muscle), while only about 900 per cubic millimetre in the rat tibialis anterior (fast muscle) (Schmalbruch and Hellhammer 1977).

Factors that govern satellite cell number have not been identified, but they are seemingly expressed during muscle maturation, since there is little difference between muscles at birth. Fibre size per se is probably not critical nor are metabolic properties, as certain type IIB—glycolytic—myofibers have as many satellite cells as oxidative myofibers (Kelly 1978).

The number of satellite cells is also muscle specific. For example, the masseter has fewer satellite cells than the extensor digitorum longus, despite the fact that these two muscles have similar fibre composition (Muller et al. 2001; Ono et al. 2010). In addition, the behaviour of satellite cells varies from one muscle to another. For example soleus-derived cells are able to differentiate earlier than extensor digitorum longus-derived satellite cells (Ono et al. 2010). Masseter-derived myoblasts proliferate faster than limb muscle myoblasts: after 3 days of culture, approximately 75 % of limb muscle myoblasts do not present a phenotype corresponding to differentiation compared with 12 % for soleus myoblasts that are already differentiated at that time point. Low numbers of satellite cells that proliferate longer prior to differentiation may contribute to the poor regenerative capacity of the masseter in response to acute damage, compared to the efficient regeneration of limb muscles.

Following these observations, we can hypothesise that satellite cells with different properties may be remobilized for different purposes. Satellite cells that have limited proliferative capacity may be oriented towards homeostatic functions that require the replacement of few myonuclei. Muscle regeneration, however, requires the synchronous activation and rapid expansion of a wide pool of satellite cells to generate thousands of myonuclei. While also self-renewing to maintain a viable stem cell compartment able to respond to future trauma for effective repair (Ono et al. 2010). Muscle structure, nature, and environment could therefore regulate satellite cell number and determine satellite cell activity.

2 From Active Myoblast Divisions in Developing Animals to Quiescence in Young Adults

Mononucleated myogenic cells are most abundant during the perinatal period after birth, when they contribute in large numbers to muscle growth (Gnocchi et al. 2009). Subsequently, their number drops in 2 waves. Satellite cells first undergo a rapid post-natal decline (loss of 32 %–5 % of sublaminal nuclei) when myogenic cells begin to enter cellular quiescence from about 14 days after birth in the mouse. This event lasts up to 2 months for the mouse and up to 9 years for the human (Cardasis and Cooper 1975; Saito 1985; Goldring et al. 2002). The decline in cell number correlates with an increase in myonuclei attributed to the fusion of juvenile satellite cells following their differentiation. The second decline of satellite cells occurs gradually, during ageing (Collins et al. 2005). These cells then possess a more limited proliferative potential (Snow 1977; Blau and Webster 1981). It has been reported that the cellular niche plays an important role in ageing and impinges on satellite cells function (Gopinath and Rando 2008).

Satellite cells proliferate in growing muscle (Shafiq et al. 1968), and S phase labelling using tritiated thymidine suggests that satellite cells give rise to myonuclei following cell division (Moss and Leblond 1970). DNA replication tracking indicates that satellite cell divisions can be asymmetric in growing muscle and thus give rise to both myonuclei and satellite cells, further suggesting that satellite cells self-renew (Moss and Leblond 1971). This data puts forth the following scenario: during the post-natal period, fast-dividing satellite cells undergo asymmetric divisions to produce both myonuclei for muscle growth and new satellite cells. Later however, these cells also undergo symmetric divisions, in order to maintain a pool of fast dividing satellite cells in the adult muscle.

In this context, it is worthwhile to mention the quantal mitosis model for regulating skeletal muscle cell division prior to differentiation and fusion. This model proposes that once a stem cell is committed, it carries out a set number of symmetrical and obligate divisions to produce differentiated myoblasts (Quinn et al. 1985). This concept was introduced by H. Holtzer and described mitotic events that yield daughter cells with metabolic properties distinct from those of the mother cell. Quantal mitosis hence contrasts with proliferative mitosis, in which daughter cells are identical to the mother cell. H. Holtzer's model implies that changes in cell determination that occur throughout development take place during these special quantal mitoses (Holtzer et al. 1975). Another model proposes that a heterogeneous population of presumptive myoblasts is produced in the absence of a quantal cell cycle. According to this model, cells would actively divide and then would stop dividing and fusion (Buckley and Konigsberg 1977; Lee et al. 1984).

Myogenesis has been shown to persist longer in severe injury than in minor injury (Grounds and McGeachie 1987). Grounds and McGeachie studied the initiation of muscle precursor replication as well as the duration of proliferation following adult skeletal muscle injury in mice. Autoradiographic detection of injected tritiated thymidine ^{3}H-Thy was used to determine the number of divisions

performed by muscle precursors before fusing to form myotubes in vivo (Grounds and McGeachie 1987). The heterogeneity of ^{3}H-Thy incorporation in the mononuclei observed was not compatible with quantal mitosis cell division. Grounds and McGeachie also found that cells divide at least 3–4 times before fusing (Grounds and McGeachie 1987).

These findings should be further considered in the context of the participation of different cell populations in myogenesis. If satellite cells alone give rise to myonuclei, the number of myoblast divisions is not predetermined and cells will divide until they fuse. However, if other cell types participate in myogenesis, these two models would need to be reinterpreted.

Another possibility considering the stem cell nature of satellite cells is that satellite cells are "paused" myoblasts (Mauro 1961) with limited tissue regeneration potential. If such is the case, does a proportion of these myoblasts then become specified as satellite cells by the satellite cell niche, thereby explaining the heterogeneity of the satellite cell population? The enforcement of a stem cell fate in a tissue-committed cell type has already been described in another system, namely in the ovaries of adult *Drosophila melanogaster* (Kai and Spradling 2004). Germinal stem cells (GSCs) remain undifferentiated as long as they are in contact with *Decapentaplegic* (Dpp) ligand signalling sources (in the niche when in contact with cap cells). Furthermore, when proliferating cystocytes which are derived from GSCs, come to contact with Dpp ligand, they revert to functional GSCs in both larvae and adult *Drosophila*. Alternatively, the muscle niche might initially be occupied by a mix of foetal myoblasts and dedicated satellite cells. Either these satellite cells alone give rise to *bona fide* adult satellite cells, either all myoblasts do, implying that they possess equal potential to generate satellite cells.

Although they actively divide during growth and after injury, satellite cells are quiescent during adulthood.

3 The Dynamics of Adult Quiescent Satellite Cells

The first evidence of the quiescence of adult muscle satellite cells comes from electron microscopy studies that detected only a small number of satellite cells (and not the myonuclei) incorporating injected ^{3}H-thymidine (Schultz et al. 1978). A subsequent study showed that after transplantation of ^{3}H-thymidine treated EDL into the muscle bed of an untreated animal, the myonuclei of the host became ^{3}H-thymidine labelled. This data suggests that satellite cells are remobilized to multiply and contribute to muscle repair in response to tissue damage (in this case triggered by the transplant).

The molecular basis of quiescence, which is important to retain the proliferative and differentiative potential of satellite cells throughout life, is beginning to be understood. Studies suggest that myostatin, a skeletal muscle specific TGF-β family member, suppresses satellite cell activation (McCroskery et al. 2003). Myostatin was also shown to induce a cyclin-dependent kinase inhibitor, p21, in vitro

(Thomas et al. 2000). Genome-wide gene expression analyses of purified satellite cells sorted by FACS have established a detailed signature of quiescence in these cells (Fukada et al. 2007; Pallafacchina et al. 2010). Negative cell cycle regulators such as the cyclin-dependent kinase inhibitors p21 and p57 are up-regulated. The self-renewing Pax7 gene and Spry1 (tyrosine kinase receptor signalling inhibitor required for the return to quiescence) (Abou-Khalil et al. 2009) are also up-regulated. Myogenic inhibitors such as musculin/MyoR are down-regulated during differentiation (MyoR antagonises MyoD activity) (Lu et al. 1999). Notch3, a key player in satellite cells activation is also up-regulated inhibiting myoblast differentiation by repressing MyoD (Kuroda et al. 1999). Notably, Notch3 was found in half of Pax7 positive cells on section (Fukada et al. 2007) and on isolated single fibres by immunostaining. Furthermore, BMP4 is up-regulated in satellite cells and this diffusible factor is known to down-regulate MyoD in somites (Reshef et al. 1998). Interestingly, positive regulators of Myf5 and Pax7, Gli2, and Meox2 respectively (Gustafsson et al. 2002; Mankoo et al. 2003), were also found to be up-regulated in quiescent cells.

Additionally, quiescent satellite cells express cell–cell adhesion molecules such as VCAM-1, N-CAM, CD34, and Esam at higher levels than non-quiescent cells (Fukada et al. 2007). This is worth noting, as the extracellular matrix and cell–cell adhesion are thought to play an important role in maintaining satellite cells in a quiescent or undifferentiated state. By using isolated myofibers as a model of satellite cell activation, Beauchamp et al. showed that quiescent satellite cells express CD34 and that an early feature of their activation is alternate splicing followed by complete transcriptional shutdown of CD34. This data implicates CD34 in the maintenance of satellite cell quiescence (Beauchamp et al. 2000).

The quiescent state appears to be associated with enhanced stress resistance (Gnocchi et al. 2009). Notably, a group of genes that confer resistance to xenobiotics and oxidative stress were found induced during quiescence (Pallafacchina et al. 2010). These include genes that encode efflux channels of the multidrug resistance family that pump toxic substances out of the cell, as well as members of the cytochrome P450 family or flavins, which are involved in the solubilisation of toxins by hydroxylation, and enzymes such as Gpx3, responsible for mitigating reactive oxygen species-mediated cytotoxicity.

4 Satellite Cells Can Enter Dormancy in Absence of Oxygen and in Cadavers

4.1 Description of the Phenomenon

Cadaver stem cells are already used in various experimental practices (Blazar et al. 1986; Kapelushnik et al. 1998; Palmer et al. 2001; Machalinski et al. 2003; Liu et al. 2006; Erker et al. 2010; Latil et al. 2012). Histological sections of biopsy

sampled on cadavers up to 17 days post-mortem showed that despite muscle necrosis, satellite cells (detected using CD56 and NCAM antibodies) remain in a relatively good shape (Latil et al. 2012). To test whether cadaver stem cells were functional, the biopsy samples were digested and plated in vitro. Four days post-plating, some cells showed divisional capacity, and these cells fused to form new myofibers. Similar results were obtained in vivo by Latil et al. Digested muscle grafted to immunosuppressed mice showed engraftment capacities. The cells were able to amplify, differentiate, and self-renew to insure the maintenance of a stem cell pool (Latil et al. 2012). These results show that after an extended period of time, cadaver satellite cells are still alive and functional. Interestingly all other differentiated cells (interstitial, fibroblasts) were dead and could not be amplified post-mortem, suggesting a selective resistance of stem cells. These findings were confirmed using mice models, in which 70 % of the satellite cells could be isolated and remained functional up to 8 days post-mortem. The same results were obtained for hematopoietic stem cells (HSC) isolated from cadaver bone marrow. These HSC were still alive and functional, able to reconstitute all the blood lineages (Latil et al. 2012).

As previously described, quiescent satellite cells receive many signals from the niche. Within cadavers, the cells display a new phenotype: they seem dormant. Indeed histological analyses show that these cells' cytoplasm is much smaller than that of their quiescent counterparts.

4.2 Mobility of Dormant Cells

Although there is no doubt as to myoblasts' mobility, whether or not satellite cells are capable of high mobility is not completely clear. Quiescent satellite cells are activated upon injury, and they can travel considerable distances within the muscle. In a focal crush injury, satellite cells located several millimetres from the site of injury are stimulated to proliferate and migrate towards the site of injury, probably while still located under the basement membrane (Schultz et al. 1988). Grafting larger muscles, which implies separating the muscle from the adjacent nerves, tendons, and blood vessels, results in a central area of ischemic necrosis where satellite cells migrate from beneath the basement membrane of degenerating myofibers towards the distal part of the muscle (Schultz et al. 1988). During the subsequent revascularization of the newly formed muscle, satellite cells migrate back to the centre of the fibre to participate in myogenesis. In a freeze injury model, during which the muscle of the anaesthetized animal is frozen and gives rise to a "dead zone" and an "alive zone", the necrotic zone is repopulated by satellite cells migrating from the viable zone (Phillips et al. 1990; Gayraud-Morel et al. 2009).

Migration across the basement membrane has also been observed in juvenile rats following in vivo ^{3}H-thymidine injection. Labelled nuclei were found in apparently undamaged myofibers away from the site of injection (Grounds et al. 2002).

The local application of myotoxic venoms and other stress-inducing substances induces translaminal migration of satellite cells from undamaged myofibers. Capturing those cells while crossing the basement membrane and migrating is difficult, but a distinct separation between the cell and myofiber has been detected (Snow 1977; Salleo et al. 1983; Schultz et al. 1988).

In addition to migrating from one fibre to another, satellite cells are able to move from one muscle to an adjacent one. A series of experiments using genetic variants of metabolic enzymes to identify donor and receptor tissues in adult mice showed that myogenic cells from host muscles invade grafted, regenerating muscle in their proximity (Grounds et al. 1980; Morgan et al. 1987; Watt et al. 1987). Similar experiments in the rat, however, failed to reproduce these results (Ghins et al. 1984) and it was suggested that the thicker epimysium of the rat could block the invasion. These findings raise the possibility that a damaged epimysium might allow transmuscle invasion by satellite cells (Schultz et al. 1986).

The current challenge is to identify the effector molecules and pathways involved in the regulation of the satellite cell motility. Recently, Siegel et al. used live video-microscopy to study the behaviour of primary satellite cells extracted and plated on different substrates. Their observations found that satellite cells are most motile on laminin. The authors also revealed an important role for hepatocyte growth factor (HGF) in promoting directional persistence, likely via the Met receptor, which is reportedly expressed by satellite cells (Siegel et al. 2009). Distinct from simple motility, directed migration and pathfinding are mediated by the activity of cell surface guidance receptors, whose signalling results in attraction or repulsion. These receptors might also play a role in contact-mediated signalling, which is distinct from migration guidance. It has been suggested that cell migration is necessary for cell aggregation and fusion to form myotubes (Kuang et al. 2007). Interestingly, while adherence molecules have not been examined in the context of myoblast migration, several of these molecules, such as NCAM, seem necessary for myogenic differentiation in vitro (Charlton et al. 2000).

Taken together, these findings suggest that satellite cells have an extensive motile capacity both in vivo and in vitro. After muscle injury these cells may travel long distances beneath the basement membrane, leave the myofiber, migrate throughout the muscle, or even reach adjacent muscle. Yet, it is important to note that in uninjured homeostatic muscle, when the basement membrane is fully formed, satellite cells probably remain in contact with the same myofiber. Siegel et al. hypothesize that HGF could be released by damaged areas of the myofiber and promote satellite cell motility, while areas of undamaged myofiber could emit repulsive signals (Siegel et al. 2009).

The results of these studies contrast with results obtained from the transplantion of dormant satellite cells (Latil et al. 2012). In this model, satellite cells show little migration capacity and often regenerate only within a limited zone adjacent to the needle track used to deliver the cells into the muscle bed.

This issue does not arise in the case of HSC, as the cells injected intravenously home to the bone marrow. The same observation was made in vitro: quiescent cells plated on matrigel, start dividing after 24 h and display high mobility few

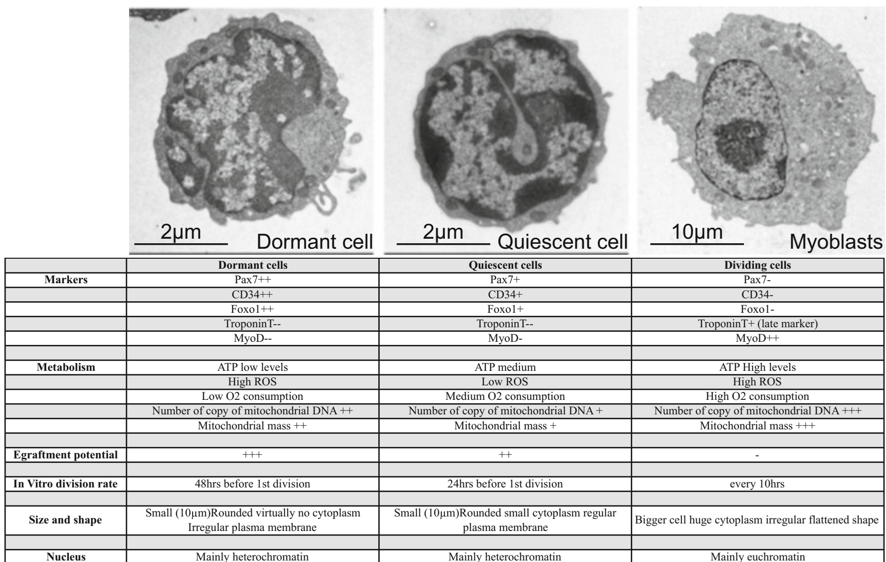
hours post-plating, but dormant cells, start dividing 48 h post-plating and their mobility is limited (Latil et al. 2012; Rocheteau et al. 2012). It is possible that once dormant, satellite cells lower their activity as much as possible, including mobility.

4.3 *Metabolism of Dormant Cells*

Proliferating and quiescent cells are expected to have different metabolic states (Fig. 1). Indeed proliferating cells must synthesise DNA, proteins, and lipids. Quiescent cells, however, do not divide and are therefore metabolically less demanding. They exhibit a decreased metabolic rate compared to their proliferative progenitors. Lymphocytes, for example, undergo a major metabolic shift when they transition between proliferation and quiescence (Bowie et al. 2006).

Mitochondria are cytoplasmic organelles found in all eukaryotic cells, and responsible for converting nutrients into adenosine triphosphate (ATP) to fuel cell activity. This process, known as aerobic respiration, is the reason why mitochondria are frequently referred to as the powerhouse of the cell. They also play a very important role in processes such as steroid metabolism, calcium homeostasis, apoptosis, and cellular proliferation.

Stem cell competence (self-renewal, differentiation, pluripotency) can be assessed using functional mitochondrial characteristics (Lonergan et al. 2006). Several mitochondrial characteristics, such as subcellular localisation, shape



	Dormant cells	Quiescent cells	Dividing cells
Markers	Pax7++	Pax7+	Pax7-
	CD34++	CD34+	CD34-
	Foxo1++	Foxo1+	Foxo1-
	TroponinT--	TroponinT--	TroponinT+ (late marker)
	MyoD--	MyoD-	MyoD++
Metabolism	ATP low levels	ATP medium	ATP High levels
	High ROS	Low ROS	High ROS
	Low O2 consumption	Medium O2 consumption	High O2 consumption
	Number of copy of mitochondrial DNA ++	Number of copy of mitochondrial DNA +	Number of copy of mitochondrial DNA +++
	Mitochondrial mass ++	Mitochondrial mass +	Mitochondrial mass +++
Egraftment potential	+++	++	-
In Vitro division rate	48hrs before 1st division	24hrs before 1st division	every 10hrs
Size and shape	Small (10μm)Rounded virtually no cytoplasm Irregular plasma membrane	Small (10μm)Rounded small cytoplasm regular plasma membrane	Bigger cell huge cytoplasm irregular flattened shape
Nucleus	Mainly heterochromatin	Mainly heterochromatin	Mainly euchromatin

Fig. 1 Summary of the main characteristics of satellite cells in anoxia (dormant cells), normoxia (satellite cells), and actively dividing cells (myoblasts). Pictures represent electron microscopy pictures of the 3-cell states

(cristae), and metabolic activity, vary with increasing passage number in culture and through spontaneous differentiation of ES cells (Facucho-Oliveira et al. 2007), justifying that these characteristics be markers of "stemness".

Undifferentiated ES cells display a perinuclear mitochondrial arrangement which is maintained in stem cells. Mitochondria adopt a more widespread cytoplasmic distribution as cells differentiate (Facucho-Oliveira et al. 2007). Furthermore, mitochondrial metabolic activity has been linked to the degree of cell differentiation: ATS cells (an adult primate stromal cell line derived from adipose tissue) show low ATP levels and a high oxygen consumption rate during the first culture passages, compared to cells after a longer cultivation period (Lonergan et al. 2006). Facucho-Oliveira et al. observed the same metabolic variations during spontaneous differentiation of ES cells (Facucho-Oliveira et al. 2007), and this was accompanied by an increase in cristae folds (increasing the surface of enzymatic reactions).

Taken together, these findings indicate that the perinuclear arrangement of mitochondria, along with a high metabolic rate and a low quantity of ATP per cell, may be good indicators of a stem cell's competence for differentiation (Fig. 1). Deriving from this profile indicates that cells are differentiating, becoming senescent, or perhaps undergoing self-renewal.

The above mentioned observations were confirmed in human haematopoietic CD34+ stem cells, in which the mitochondria, located in a mostly perinuclear fashion, displayed low oxygen consumption rates (Piccoli et al. 2005). Evidence further demonstrates that long-term haematopoietic stem cells are located in a hypoxic microenvironment (Parmar et al. 2007). Recently, it was shown that long-term HSC are metabolically adapted to survive in such an environment by using glycolysis instead of mitochondrial oxidative phosphorylation as a source of energy. This correlates with low mitochondrial activity and low expression of HIF-1, a master regulator of metabolism which regulates mitochondrial respiration and shift towards anaerobic glycolysis by transcriptional activation or up-regulation (Parmar et al. 2007; Simsek et al. 2010).

Satellite cells follow the same pattern. When quiescent, satellite cells display low mitochondrial activity, thus limiting the production of ROS (Reactive Oxygen Species) which can be detrimental for the cell, and preferentially use glycolysis over oxidative phosphorylation (Lunt and Vander Heiden 2011).

Notch is known to interact with HIF1a. HIF1a expression increases in hypoxic environments, subsequently up-regulating Notch targets. This up-regulation has been shown to prevent differentiation and to force cells to remain quiescent. The direct role of HIF-1a in maintaining quiescence is illustrated by studies on HSCs. In these studies, hypoxic conditions lead to transcriptional up-regulation and an activation of many HIF1a target sequences, including those controlling genes that play a key role in cell cycle regulation and survival (Takubo et al. 2010). In hypoxic conditions, the phosphoinositide 3-kinase–Akt signalling pathway is down-regulated in correlation with reduced sensitivity of the IGF receptor to growth factors. Premature differentiation of muscle stem cells is thus prevented. The Akt pathway activates mammalian target of rapamycin (mTOR), which is involved in

nutrient response and high-energy metabolism. When nutrients availability is low, kinase Ulk1 is activated, activating autophagy. mTOR prevents this activation. In quiescent satellite cells, *Ulk1* is expressed at high levels, favouring autophagy. A concomitant down-regulation of mTOR is important to prevent senescence and there is now evidence, mainly from work on HSCs, that autophagy also is critical to prevent senescence, by helping maintain mitochondrial quality and viability in quiescent cells (Valcourt et al. 2012).

These conditions (low metabolic activity, anaerobic metabolism, autophagy) could explain why such cells can enter dormancy post-mortem and do not die directly. The dormant state has indeed been associated with an even lower metabolic rate than the quiescent state. Dormant cells consume 28 % less oxygen than their quiescent counterparts, and their oxygen consumption remains low for extended periods of time (Fig. 1, Latil et al. 2012). Accordingly, ATP levels are much lower in dormant cells when compared with quiescent cells. In cadavers, after an initial decrease, ATP levels remain constant, as if a basal state were attained, allowing the cells to survive in unfavourable conditions (Latil et al. 2012). The cellular redox ratio NAD+/NADH, reflecting cell health and metabolism (Ying 2008) stays constant in satellite cells post-mortem, indicating healthy cells despite counter intuitively an eightfold increase in ROS content. This observation asks for further investigation because although high levels of ROS are generally interpreted as detrimental to the cell it has also been shown that increased ROS can serve as a differentiation signal in HSCs (Jang and Sharkis 2007) and is even found to promote survival in *C.elegans* (Gruber et al. 2011). Higher levels of ROS together with a constant redox state could characterise dormant satellite cells.

As for mitochondrial state, dormant cells show a 30 % decrease in mitochondrial mass (Fig. 1). Nevertheless, 3D analysis reveals that the overall organisation of the mitochondrial network is comparable to the network of quiescent cells (Latil et al. 2012). Decreased transcription, including that of *Sod* genes (Super-Oxide-Dismutase; specific mitochondrial genes) strengthens these observations and links them to previous studies reporting a decline in mitochondrial RNAs and SOD enzymes in a low-oxygen environment (Van Itallie et al. 1993; Son et al. 2008). Although mitochondrial mass and transcriptional activity decreases, mitochondrial DNA increases notably (by 58 %) in post-mortem satellite cells (Latil et al. 2012). The increase in mitochondrial DNA content could be attributed to several non-exclusive factors. Activation of cell cycle checkpoints, phosphorylation of cell cycle arrest kinase 2 Chk2 which triggers an increase in mitochondrial DNA independently from TFAM (a regulator of the number of mitochondrial DNA) (Niu et al. 2012) and/or the oxidative stress response which is known to increase mitochondrial DNA could be at play (Mecocci et al. 1993; Lee et al. 2000; Al-Kafaji and Golbahar 2013).

4.4 Molecular Characterisation of Dormant Cells, Gene Expression

Pax genes play a critical role in tissue and organ formation during embryogenesis and regeneration in the adult. The Pax gene family encodes a group of transcription factors that are characterised by a paired box domain. This domain allows sequence-specific DNA binding. In addition, some members of the Pax family possess an octapeptide motif and/or a partial or complete homeobox DNA-binding domain (Chi and Epstein 2002; Buckingham and Relaix 2007). *Pax7* is currently the most reliable marker of adult satellite cells (Seale et al. 2000; Zammit et al. 2004) used to identify all post-natal satellite cells in animals. Satellite cells in some muscles such as the diaphragm also express *Pax3*. However, Pax3 only identifies a subset of satellite cells in late foetal stages and does so in a subset of muscles including the diaphragm and some limb muscles (Relaix et al. 2006). Studies disagree on the effect of *Pax 3/7 overexpression on* satellite cell differentiation in the adult. For example, high Pax3 levels have been reported to inhibit satellite cell differentiation (Olguin and Olwin 2004), whereas lower Pax3 levels allow differentiation to proceed unhindered (Relaix et al. 2006). But the main *Pax* gene expressed in satellite cells is *Pax7*. In perinatal satellite cells *Pax7* was reported to play a critical role in cell survival. *Pax7* mutant mice rapidly lose these cells to apoptosis after birth (Seale et al. 2000). Consistent with a role for Pax7 in muscle stem/progenitor survival, Lepper et al. demonstrated that the expression of a dominant negative form of Pax7 in cultured satellite cells promotes high levels of apoptosis and significant loss of myogenic cells. This study also showed that inactivation of Pax7 at P7 (7 days after birth) results in a depletion of juvenile satellite cells due to accelerated differentiation, suggesting that Pax7 delays the onset on differentiation to allow an increase in myoblast number (Lepper et al. 2009). Little is known about the role of *Pax7* in muscle cell proliferation, but *Pax7* mutant mice exhibit compromised satellite cell proliferation (Oustanina et al. 2004), and an extension of the G2/M cell cycle phase in these cells (Relaix et al. 2006). Pax7 plays a role in migration and seemingly induces changes in cell size and morphology. Indeed, constitutive Pax7 expression results in smaller, rounder cells, a phenotype attributed to stem-like cells. Conversely, dominant-negative inhibition of *Pax7* results in increased cell size. Such cell shape changes are independent from the myogenic programme, as they are observed in NIH 3 T3 mouse fibroblasts (Collins et al. 2009).

The myogenic regulatory factors (MRFs) are an evolutionary conserved family of four bHLH transcription factors with regulatory functions in muscle progenitor cell determination (MyoD, Myf5, and MRF4) and differentiation (Myogenin). These factors determine muscle identity and activate differentiation markers. Myogenin is a key marker for the onset of differentiation, although the inactivation of this gene does not systematically compromise differentiation.

MyoD was the first MRF to be discovered using a molecular screen of genes with dominant regulatory activities on 10 T1/2, embryonic fibroblasts conversion to

stable myoblasts. MyoD was also the first MRF to be cloned using a single cDNA transfection in 10 T1/2 (Davis et al. 1990). *Myf5*, *Myogenin*, and *Mrf4* were then identified based on sequence homology with MyoD or by using functional screens (Pownall et al. 2002). MyoD is a protein with a key role in regulating muscle cell fate and differentiation and acts sequentially in myogenic differentiation. It is one of the earliest markers of myogenic commitment. MyoD protein is expressed in activated but not in quiescent satellite cells (Gu et al. 1993). Although MyoD null animals are viable, some null mutants die at birth, indicating an interplay with other undefined factors like the genetic backgound of the mouse (Rudnicki et al. 1992).

Myf5 is up-regulated at early stages of satellite cell activation. In Myf5 null animals, early regeneration is delayed and mutant satellite cells display a minor proliferation defect under certain conditions (Gayraud-Morel et al. 2007). Unlike MyoD, Myf5 fails to promote differentiation in *Mrf4:Myod* or *Mrf4:Myod: Myogenin* mutants (Rawls et al. 1998; Valdez et al. 2000) suggesting that Myf5 is not directly involved in the decision to differentiate per se. After injury in Myf5 null mice, there is no change in satellite cell number but there is an increase in muscle fibre diameter (hypertrophy) and presence of adipocytes in the regenerating muscle bed depending on the injury model studied (Gayraud-Morel et al. 2007).

Myogenin is required for the functional development of skeletal muscle. The expression of other MRFs cannot compensate for a Myogenin deficiency in the embryo. Myogenin targets are mainly structural genes such as components of the z-line, which serve as anchor points to the sarcomere (the basic contractile unit of the muscle) (Davie et al. 2007). When the gene is conditionally removed using a floxed *Myogenin* allele, myofibers after birth are smaller. *Myogenin* is thus required for achieving normal body size, but not for skeletal muscle growth during post-natal life (Meadows et al. 2008). The role of this factor in muscle regeneration following acute injury has yet to be established, but Myogenin clearly reflects a differentiation state of the cell.

MRF4 (or Myf6) is not expressed in quiescent satellite cells, but is expressed at very high levels in the nuclei of mature adult muscle (Gayraud-Morel et al. 2007). In the adult, studies suggest that mRNA levels of MRF4 vary highly dependent on fibre type (Walters et al. 2000), pointing to a potential role of MRF4 in fibre type maturation.

A late marker of differentiation is TroponinT which is part of the troponin complex. It binds the tropomyosin complex to form a troponin–tropomyosin complex. Troponin T binding to tropomyosin enables its physical interaction with actin and consequently mediates striated muscle contraction (Gordon et al. 2000). Myosin heavy chain (MyHC) is one of the major components of the contractile apparatus of all striated muscles. The functional heterogeneity of muscles can be explained by the different MyHC isoforms (Bottinelli 2001). Various factors like development, innervation, increased and decreased neuromuscular activity, physical activity, and ageing, influence skeletal muscle fibre phenotype (Schiaffino and Reggiani 1996; Pette and Staron 2000). Embryonic and perinatal isoforms are expressed during development and reexpressed during muscle regeneration (Mahdavi et al. 1986). The other heavy chain form is expressed in adult muscles.

Quiescent satellite cells express *Pax7*, *CD34*, and 90 % express *Myf5* (Kuang et al. 2007). Virtually no quiescent satellite cells express *MyoD or* Myogenin. Some quiescent cells do however express *MyoD* at the RNA level and although they remain quiescent, Rocheteau et al. have shown that these cells are more likely to differentiate and undergo faster activation (Rocheteau et al. 2012). Quiescent satellite cells are thus primed to the muscle lineage but do not express any genes responsible for differentiation.

Dormant satellite cells 4–8 days post-mortem, down-regulate commitment markers Myogenin and TroponinT and up-regulate genes associated with the cell "stem" state (Pax7 and CD34, Fig. 1) (Latil et al. 2012). This suggests that post-mortem-derived muscle stem cells are less transcriptionally primed for myogenic commitment and thus adopt a more stem-like state. In the dormant state and in accordance with previous findings, a number of genes involved in oxidative stress, hypoxia, and apoptosis are up-regulated. Such genes include antioxidant glutathione peroxidase 1, cell cycle regulator p21, Foxo1—a gene involved in survival during oxidative stress (Diehn et al. 2009)—hypoxia-inducible factor 3a2—a transcription factor that regulates adaptive responses to hypoxia (Majmundar et al. 2010)—and angiopoietin 1, which promotes muscle stem cell quiescence (Abou-Khalil et al. 2009). Although high levels of ROS activate Nf-κb (Gloire et al. 2006), no Nf-κb was detected post-mortem using reporter mice (Latil et al. 2012). Taken together, these results indicate that post-mortem satellite cells less committed to the myogenic lineage adopt a more stem like state and display specific stem state markers. The increase of *Pax7* expression is coherent with the absence of increase in cell apoptosis in deleterious post-mortem conditions. Higher expression of genes that control the cell cycle concords with the increased time it takes for dormant cells to exit the cell cycle.

4.5 Quiescence Is a Prerequisite for Cell Entry into Dormancy

After injury in the adult, satellite cells are mobilised: they activate, divide and fuse to regenerate myotubes.

During satellite cell activation *Calcitonin* receptor expression is rapidly down-regulated. This receptor was shown to inhibit the activation of quiescent satellite cells via cAMP signalling (Becker et al. 2004; Fukada et al. 2007). Another intrinsic signal for satellite cell activation is the sphingosine-1-phosphate located on the plasma membrane. Sphingosine-1-phosphate inhibition dramatically compromises muscle regeneration (Nagata et al. 2006).

Extrinsic signalling from the niche also plays an important role in satellite cell activation. For example, mechanical stretching of the fibre activates nitric oxide synthesis, which in turn triggers the release of HGF. HGF binds the Met receptor and activates the satellite cell (Wozniak and Anderson 2007). Nitric oxide also

induces the expression of Follistatin that inhibits Myostatin, a negative regulator of myogenesis (Pisconti et al. 2006) and could thus help satellite cells exit quiescence.

Growth factors diffusing from the microenvironment constitute a third stimulus for satellite cell exit from quiescence. FGF for example was shown to induce pro-myogenic MAPK signalling cascades, which are required for satellite cell activation and the regulation of quiescence (Jones et al. 2005).

Satellite cells only enter dormancy post-mortem if they are quiescent at the time of their host's death. When activated or in mitosis, satellite cells do not survive and do not enter dormancy (Latil et al. 2012). Whether it is the intrinsic status of the cell (metabolic state and ROS levels too high, genes repressing this state) or extrinsic signals, such as those previously mentioned that prevent the cell from becoming dormant has yet to be determined. What is certain is that the presence of NO and glucose deprivation (which occur post-mortem) decrease cell viability (Beltran et al. 2000). Also, HGF, released upon injury, leads to a mitochondrial depolarization that can trigger apoptosis via Mimp (a mitochondrial carrier) (Yerushalmi et al. 2002).

Concluding Remarks

Satellite cells have great potential in regenerative medicine to cure or improve the condition of individuals suffering from muscular degenerative diseases. However, these cells' limited availability and low mobility imply that a great number of injections all over the body be required for efficient therapy and therefore constitute barriers to their present use in therapeutics. The finding that those cells can enter a "dormant" state is promising for two reasons. First, this new muscle satellite cell state could represent a subpopulation of cells that are deeply quiescent and characterising dormant cells could lead us to discover new properties that could be used in medicine. Second, the possible use of cells from cadavers (like organs nowadays) could counteract satellite cells' limited availability. Thus, a deeper understanding of the mechanisms which control satellite cell "stemness", quiescence, dormancy, and activation is key to further their potential as therapeutics. Further experiments are needed in order to determine whether dormancy is a feature of a specific subpopulation of cells or if this state actually exists in vivo, *and* to develop a better understanding of the factors and mechanisms that are key to dormancy.

References

Abou-Khalil R, Brack AS (2010) Muscle stem cells and reversible quiescence: the role of sprouty. Cell Cycle 9(13):2575–2580

Abou-Khalil R, Le Grand F, Pallafacchina G, Valable S, Authier FJ, Rudnicki MA, Gherardi RK, Germain S, Chretien F, Sotiropoulos A et al (2009) Autocrine and paracrine angiopoietin 1/Tie-2 signaling promotes muscle satellite cell self-renewal. Cell Stem Cell 5(3):298–309

Al-Kafaji G, Golbahar J (2013) High glucose-induced oxidative stress increases the copy number of mitochondrial DNA in human mesangial cells. Biomed Res Int 2013:754946

Beauchamp JR, Heslop L, Yu DS, Tajbakhsh S, Kelly RG, Wernig A, Buckingham ME, Partridge TA, Zammit PS (2000) Expression of CD34 and Myf5 defines the majority of quiescent adult skeletal muscle satellite cells. J Cell Biol 151(6):1221–1234

Becker KL, Nylen ES, White JC, Muller B, Snider RH Jr (2004) Clinical review 167: Procalcitonin and the calcitonin gene family of peptides in inflammation, infection, and sepsis: a journey from calcitonin back to its precursors. J Clin Endocrinol Metab 89(4):1512–1525

Beltran B, Mathur A, Duchen MR, Erusalimsky JD, Moncada S (2000) The effect of nitric oxide on cell respiration: A key to understanding its role in cell survival or death. Proc Natl Acad Sci U S A 97(26):14602–14607

Blau HM, Webster C (1981) Isolation and characterization of human muscle cells. Proc Natl Acad Sci U S A 78(9):5623–5627

Blazar BR, Lasky LC, Perentesis JP, Watson KV, Steinberg SE, Filipovich AH, Orr HT, Ramsay NK (1986) Successful donor cell engraftment in a recipient of bone marrow from a cadaveric donor. Blood 67(6):1655–1660

Bottinelli R (2001) Functional heterogeneity of mammalian single muscle fibres: do myosin isoforms tell the whole story? Pflugers Arch 443(1):6–17

Bowie MB, McKnight KD, Kent DG, McCaffrey L, Hoodless PA, Eaves CJ (2006) Hematopoietic stem cells proliferate until after birth and show a reversible phase-specific engraftment defect. J Clin Invest 116(10):2808–2816

Buckingham M, Relaix F (2007) The role of Pax genes in the development of tissues and organs: Pax3 and Pax7 regulate muscle progenitor cell functions. Annu Rev Cell Dev Biol 23:645–673

Buckley PA, Konigsberg IR (1977) Do myoblasts in vivo withdraw from the cell cycle? A reexamination. Proc Natl Acad Sci U S A 74(5):2031–2035

Cardasis CA, Cooper GW (1975) An analysis of nuclear numbers in individual muscle fibers during differentiation and growth: a satellite cell-muscle fiber growth unit. J Exp Zool 191 (3):347–358

Charlton CA, Mohler WA, Blau HM (2000) Neural cell adhesion molecule (NCAM) and myoblast fusion. Dev Biol 221(1):112–119

Chi N, Epstein JA (2002) Getting your Pax straight: Pax proteins in development and disease. Trends Genet 18(1):41–47

Collins CA, Olsen I, Zammit PS, Heslop L, Petrie A, Partridge TA, Morgan JE (2005) Stem cell function, self-renewal, and behavioral heterogeneity of cells from the adult muscle satellite cell niche. Cell 122(2):289–301

Collins CA, Gnocchi VF, White RB, Boldrin L, Perez-Ruiz A, Relaix F, Morgan JE, Zammit PS (2009) Integrated functions of Pax3 and Pax7 in the regulation of proliferation, cell size and myogenic differentiation. PLoS One 4(2):e4475

Davie JK, Cho JH, Meadows E, Flynn JM, Knapp JR, Klein WH (2007) Target gene selectivity of the myogenic basic helix-loop-helix transcription factor myogenin in embryonic muscle. Dev Biol 311(2):650–664

Davis RL, Cheng PF, Lassar AB, Weintraub H (1990) The MyoD DNA binding domain contains a recognition code for muscle-specific gene activation. Cell 60(5):733–746

de Maruenda EC, Franzini-Armstrong C (1978) Satellite and invasive cells in frog sartorius muscle. Tissue Cell 10(4):749–772

Diehn M, Cho RW, Lobo NA, Kalisky T, Dorie MJ, Kulp AN, Qian D, Lam JS, Ailles LE, Wong M et al (2009) Association of reactive oxygen species levels and radioresistance in cancer stem cells. Nature 458(7239):780–783

Erker L, Azuma H, Lee AY, Guo C, Orloff S, Eaton L, Benedetti E, Jensen B, Finegold M, Willenbring H et al (2010) Therapeutic liver reconstitution with murine cells isolated long after death. Gastroenterology 139(3):1019–1029

Facucho-Oliveira JM, Alderson J, Spikings EC, Egginton S, St John JC (2007) Mitochondrial DNA replication during differentiation of murine embryonic stem cells. J Cell Sci 120 (Pt 22):4025–4034

Fukada S, Uezumi A, Ikemoto M, Masuda S, Segawa M, Tanimura N, Yamamoto H, Miyagoe-Suzuki Y, Takeda S (2007) Molecular signature of quiescent satellite cells in adult skeletal muscle. Stem Cells 25(10):2448–2459

Gayraud-Morel B, Chretien F, Flamant P, Gomes D, Zammit PS, Tajbakhsh S (2007) A role for the myogenic determination gene Myf5 in adult regenerative myogenesis. Dev Biol 312(1):13–28

Gayraud-Morel B, Chretien F, Tajbakhsh S (2009) Skeletal muscle as a paradigm for regenerative biology and medicine. Regen Med 4(2):293–319

Ghins E, Colson-van Schoor M, Marechal G (1984) The origin of muscle stem cells in rat triceps surae regenerating after mincing. J Muscle Res Cell Motil 5(6):711–722

Gibson MC, Schultz E (1982) The distribution of satellite cells and their relationship to specific fiber types in soleus and extensor digitorum longus muscles. Anat Rec 202(3):329–337

Gloire G, Legrand-Poels S, Piette J (2006) NF-kappaB activation by reactive oxygen species: fifteen years later. Biochem Pharmacol 72(11):1493–1505

Gnocchi VF, White RB, Ono Y, Ellis JA, Zammit PS (2009) Further characterisation of the molecular signature of quiescent and activated mouse muscle satellite cells. PLoS One 4(4): e5205

Goldring K, Partridge T, Watt D (2002) Muscle stem cells. J Pathol 197(4):457–467

Gopinath SD, Rando TA (2008) Stem cell review series: aging of the skeletal muscle stem cell niche. Aging Cell 7(4):590–598

Gordon AM, Homsher E, Regnier M (2000) Regulation of contraction in striated muscle. Physiol Rev 80(2):853–924

Grounds MD, McGeachie JK (1987) A model of myogenesis in vivo, derived from detailed autoradiographic studies of regenerating skeletal muscle, challenges the concept of quantal mitosis. Cell Tissue Res 250(3):563–569

Grounds M, Partridge TA, Sloper JC (1980) The contribution of exogenous cells to regenerating skeletal muscle: an isoenzyme study of muscle allografts in mice. J Pathol 132(4):325–341

Grounds MD, White JD, Rosenthal N, Bogoyevitch MA (2002) The role of stem cells in skeletal and cardiac muscle repair. J Histochem Cytochem 50(5):589–610

Gruber J, Ng LF, Fong S, Wong YT, Koh SA, Chen CB, Shui G, Cheong WF, Schaffer S, Wenk MR et al (2011) Mitochondrial changes in ageing Caenorhabditis elegans–what do we learn from superoxide dismutase knockouts? PLoS One 6(5):e19444

Gu W, Schneider JW, Condorelli G, Kaushal S, Mahdavi V, Nadal-Ginard B (1993) Interaction of myogenic factors and the retinoblastoma protein mediates muscle cell commitment and differentiation. Cell 72(3):309–324

Gustafsson MK, Pan H, Pinney DF, Liu Y, Lewandowski A, Epstein DJ, Emerson CP Jr (2002) Myf5 is a direct target of long-range Shh signaling and Gli regulation for muscle specification. Genes Dev 16(1):114–126

Guth L (1973) Fact and artifact in the histochemical procedure for myofibrillar ATPase. Exp Neurol 41(2):440–450

Guth L, Yellin H (1971) The dynamic nature of the so-called "fiber types" of mammalian skeletal muscle. Exp Neurol 31(2):227–300

Holtzer H, Rubinstein N, Fellini S, Yeoh G, Chi J, Birnbaum J, Okayama M (1975) Lineages, quantal cell cycles, and the generation of cell diversity. Q Rev Biophys 8(4):523–557

Jang YY, Sharkis SJ (2007) A low level of reactive oxygen species selects for primitive hematopoietic stem cells that may reside in the low-oxygenic niche. Blood 110(8):3056–3063

Jones NC, Tyner KJ, Nibarger L, Stanley HM, Cornelison DD, Fedorov YV, Olwin BB (2005) The p38alpha/beta MAPK functions as a molecular switch to activate the quiescent satellite cell. J Cell Biol 169(1):105–116

Kai T, Spradling A (2004) Differentiating germ cells can revert into functional stem cells in Drosophila melanogaster ovaries. Nature 428(6982):564–569

Kapelushnik J, Aker M, Pugatsch T, Samuel S, Slavin S (1998) Bone marrow transplantation from a cadaveric donor. Bone Marrow Transplant 21(8):857–858

Kelly AM (1978) Perisynaptic satellite cells in the developing and mature rat soleus muscle. Anat Rec 190(4):891–903

Kuang S, Kuroda K, Le Grand F, Rudnicki MA (2007) Asymmetric self-renewal and commitment of satellite stem cells in muscle. Cell 129(5):999–1010

Kuroda K, Tani S, Tamura K, Minoguchi S, Kurooka H, Honjo T (1999) Delta-induced Notch signaling mediated by RBP-J inhibits MyoD expression and myogenesis. J Biol Chem 274 (11):7238–7244

Latil M, Rocheteau P, Chatre L, Sanulli S, Memet S, Ricchetti M, Tajbakhsh S, Chretien F (2012) Skeletal muscle stem cells adopt a dormant cell state post mortem and retain regenerative capacity. Nat Commun 3:903

Lee HU, Kaufman SJ, Coleman JR (1984) Expression of myoblast and myocyte antigens in relation to differentiation and the cell cycle. Exp Cell Res 152(2):331–347

Lee HC, Yin PH, Lu CY, Chi CW, Wei YH (2000) Increase of mitochondria and mitochondrial DNA in response to oxidative stress in human cells. Biochem J 348(Pt 2):425–432

Lepper C, Conway SJ, Fan CM (2009) Adult satellite cells and embryonic muscle progenitors have distinct genetic requirements. Nature 460(7255):627–631

Liu X, Zhu Y, Gao W (2006) Isolation of neural stem cells from the spinal cords of low temperature preserved abortuses. J Neurosci Methods 157(1):64–70

Lonergan T, Brenner C, Bavister B (2006) Differentiation-related changes in mitochondrial properties as indicators of stem cell competence. J Cell Physiol 208(1):149–153

Lu J, Webb R, Richardson JA, Olson EN (1999) MyoR: a muscle-restricted basic helix-loop-helix transcription factor that antagonizes the actions of MyoD. Proc Natl Acad Sci U S A 96 (2):552–557

Lunt SY, Vander Heiden MG (2011) Aerobic glycolysis: meeting the metabolic requirements of cell proliferation. Annu Rev Cell Dev Biol 27:441–464

Machalinski B, Paczkowski M, Kawa M, Paczkowska E, Ostrowski M (2003) An optimization of isolation of early hematopoietic cells from heparinized cadaveric organ donors. Transplant Proc 35(8):3096–3100

Mahdavi V, Strehler EE, Periasamy M, Wieczorek DF, Izumo S, Nadal-Ginard B (1986) Sarcomeric myosin heavy chain gene family: organization and pattern of expression. Med Sci Sports Exerc 18(3):299–308

Majmundar AJ, Wong WJ, Simon MC (2010) Hypoxia-inducible factors and the response to hypoxic stress. Mol Cell 40(2):294–309

Mankoo BS, Skuntz S, Harrigan I, Grigorieva E, Candia A, Wright CV, Arnheiter H, Pachnis V (2003) The concerted action of Meox homeobox genes is required upstream of genetic pathways essential for the formation, patterning and differentiation of somites. Development 130(19):4655–4664

Mauro A (1961) Satellite cell of skeletal muscle fibers. J Biophys Biochem Cytol 9:493–495

McCroskery S, Thomas M, Maxwell L, Sharma M, Kambadur R (2003) Myostatin negatively regulates satellite cell activation and self-renewal. J Cell Biol 162(6):1135–1147

Meadows E, Cho JH, Flynn JM, Klein WH (2008) Myogenin regulates a distinct genetic program in adult muscle stem cells. Dev Biol 322(2):406–414

Mecocci P, MacGarvey U, Kaufman AE, Koontz D, Shoffner JM, Wallace DC, Beal MF (1993) Oxidative damage to mitochondrial DNA shows marked age-dependent increases in human brain. Ann Neurol 34(4):609–616

Morgan JE, Coulton GR, Partridge TA (1987) Muscle precursor cells invade and repopulate freeze-killed muscles. J Muscle Res Cell Motil 8(5):386–396

Moss FP, Leblond CP (1970) Nature of dividing nuclei in skeletal muscle of growing rats. J Cell Biol 44(2):459–462
Moss FP, Leblond CP (1971) Satellite cells as the source of nuclei in muscles of growing rats. Anat Rec 170(4):421–435
Muller J, Vayssiere N, Royuela M, Leger ME, Muller A, Bacou F, Pons F, Hugon G, Mornet D (2001) Comparative evolution of muscular dystrophy in diaphragm, gastrocnemius and masseter muscles from old male mdx mice. J Muscle Res Cell Motil 22(2):133–139
Nagata Y, Partridge TA, Matsuda R, Zammit PS (2006) Entry of muscle satellite cells into the cell cycle requires sphingolipid signaling. J Cell Biol 174(2):245–253
Niu R, Yoshida M, Ling F (2012) Increases in mitochondrial DNA content and 4977-bp deletion upon ATM/Chk2 checkpoint activation in HeLa cells. PLoS One 7(7):e40572
Olguin HC, Olwin BB (2004) Pax-7 up-regulation inhibits myogenesis and cell cycle progression in satellite cells: a potential mechanism for self-renewal. Dev Biol 275(2):375–388
Ono Y, Boldrin L, Knopp P, Morgan JE, Zammit PS (2010) Muscle satellite cells are a functionally heterogeneous population in both somite-derived and branchiomeric muscles. Dev Biol 337(1):29–41
Oustanina S, Hause G, Braun T (2004) Pax7 directs postnatal renewal and propagation of myogenic satellite cells but not their specification. EMBO J 23(16):3430–3439
Pallafacchina G, Francois S, Regnault B, Czarny B, Dive V, Cumano A, Montarras D, Buckingham M (2010) An adult tissue-specific stem cell in its niche: a gene profiling analysis of in vivo quiescent and activated muscle satellite cells. Stem Cell Res 4(2):77–91
Palmer TD, Schwartz PH, Taupin P, Kaspar B, Stein SA, Gage FH (2001) Cell culture. Progenitor cells from human brain after death. Nature 411(6833):42–43
Parmar K, Mauch P, Vergilio JA, Sackstein R, Down JD (2007) Distribution of hematopoietic stem cells in the bone marrow according to regional hypoxia. Proc Natl Acad Sci U S A 104 (13):5431–5436
Pette D, Staron RS (2000) Myosin isoforms, muscle fiber types, and transitions. Microsc Res Tech 50(6):500–509
Phillips GD, Hoffman JR, Knighton DR (1990) Migration of myogenic cells in the rat extensor digitorum longus muscle studied with a split autograft model. Cell Tissue Res 262(1):81–88
Piccoli C, Ria R, Scrima R, Cela O, D'Aprile A, Boffoli D, Falzetti F, Tabilio A, Capitanio N (2005) Characterization of mitochondrial and extra-mitochondrial oxygen consuming reactions in human hematopoietic stem cells. Novel evidence of the occurrence of NAD(P)H oxidase activity. J Biol Chem 280(28):26467–26476
Pisconti A, Brunelli S, Di Padova M, De Palma C, Deponti D, Baesso S, Sartorelli V, Cossu G, Clementi E (2006) Follistatin induction by nitric oxide through cyclic GMP: a tightly regulated signaling pathway that controls myoblast fusion. J Cell Biol 172(2):233–244
Pownall ME, Gustafsson MK, Emerson CP Jr (2002) Myogenic regulatory factors and the specification of muscle progenitors in vertebrate embryos. Annu Rev Cell Dev Biol 18:747–783
Quinn LS, Holtzer H, Nameroff M (1985) Generation of chick skeletal muscle cells in groups of 16 from stem cells. Nature 313(6004):692–694
Rawls A, Valdez MR, Zhang W, Richardson J, Klein WH, Olson EN (1998) Overlapping functions of the myogenic bHLH genes MRF4 and MyoD revealed in double mutant mice. Development 125(13):2349–2358
Relaix F, Montarras D, Zaffran S, Gayraud-Morel B, Rocancourt D, Tajbakhsh S, Mansouri A, Cumano A, Buckingham M (2006) Pax3 and Pax7 have distinct and overlapping functions in adult muscle progenitor cells. J Cell Biol 172(1):91–102
Reshef R, Maroto M, Lassar AB (1998) Regulation of dorsal somitic cell fates: BMPs and Noggin control the timing and pattern of myogenic regulator expression. Genes Dev 12(3):290–303

Rocheteau P, Gayraud-Morel B, Siegl-Cachedenier I, Blasco MA, Tajbakhsh S (2012) A subpopulation of adult skeletal muscle stem cells retains all template DNA strands after cell division. Cell 148(1–2):112–125

Rudnicki MA, Braun T, Hinuma S, Jaenisch R (1992) Inactivation of MyoD in mice leads to up-regulation of the myogenic HLH gene Myf-5 and results in apparently normal muscle development. Cell 71(3):383–390

Saito Y (1985) Muscle fibre type differentiation and satellite cell population in Werdnig-Hoffmann disease. J Neurol Sci 68(1):75–87

Salleo A, La Spada G, Falzea G, Denaro MG, Cicciarello R (1983) Response of satellite cells and muscle fibers to long-term compensatory hypertrophy. J Submicrosc Cytol 15(4):929–940

Schiaffino S, Reggiani C (1996) Molecular diversity of myofibrillar proteins: gene regulation and functional significance. Physiol Rev 76(2):371–423

Schmalbruch H, Hellhammer U (1977) The number of nuclei in adult rat muscles with special reference to satellite cells. Anat Rec 189(2):169–175

Schultz E, Gibson MC, Champion T (1978) Satellite cells are mitotically quiescent in mature mouse muscle: an EM and radioautographic study. J Exp Zool 206(3):451–456

Schultz E, Jaryszak DL, Gibson MC, Albright DJ (1986) Absence of exogenous satellite cell contribution to regeneration of frozen skeletal muscle. J Muscle Res Cell Motil 7(4):361–367

Schultz E, Albright DJ, Jaryszak DL, David TL (1988) Survival of satellite cells in whole muscle transplants. Anat Rec 222(1):12–17

Seale P, Sabourin LA, Girgis-Gabardo A, Mansouri A, Gruss P, Rudnicki MA (2000) Pax7 is required for the specification of myogenic satellite cells. Cell 102(6):777–786

Shafiq SA, Gorycki MA, Mauro A (1968) Mitosis during postnatal growth in skeletal and cardiac muscle of the rat. J Anat 103(Pt 1):135–141

Siegel AL, Atchison K, Fisher KE, Davis GE, Cornelison DD (2009) 3D timelapse analysis of muscle satellite cell motility. Stem Cells 27(10):2527–2538

Simsek T, Kocabas F, Zheng J, Deberardinis RJ, Mahmoud AI, Olson EN, Schneider JW, Zhang CC, Sadek HA (2010) The distinct metabolic profile of hematopoietic stem cells reflects their location in a hypoxic niche. Cell Stem Cell 7(3):380–390

Snow MH (1977) The effects of aging on satellite cells in skeletal muscles of mice and rats. Cell Tissue Res 185(3):399–408

Son D, Kojima I, Inagi R, Matsumoto M, Fujita T, Nangaku M (2008) Chronic hypoxia aggravates renal injury via suppression of Cu/Zn-SOD: a proteomic analysis. Am J Physiol Renal Physiol 294(1):F62–F72

Takubo K, Goda N, Yamada W, Iriuchishima H, Ikeda E, Kubota Y, Shima H, Johnson RS, Hirao A, Suematsu M et al (2010) Regulation of the HIF-1alpha level is essential for hematopoietic stem cells. Cell Stem Cell 7(3):391–402

Thomas M, Langley B, Berry C, Sharma M, Kirk S, Bass J, Kambadur R (2000) Myostatin, a negative regulator of muscle growth, functions by inhibiting myoblast proliferation. J Biol Chem 275(51):40235–40243

Valcourt JR, Lemons JM, Haley EM, Kojima M, Demuren OO, Coller HA (2012) Staying alive: metabolic adaptations to quiescence. Cell Cycle 11(9):1680–1696

Valdez MR, Richardson JA, Klein WH, Olson EN (2000) Failure of Myf5 to support myogenic differentiation without myogenin, MyoD, and MRF4. Dev Biol 219(2):287–298

Van Itallie CM, Van Why S, Thulin G, Kashgarian M, Siegel NJ (1993) Alterations in mitochondrial RNA expression after renal ischemia. Am J Physiol 265(3 Pt 1):C712–C719

Walters EH, Stickland NC, Loughna PT (2000) MRF-4 exhibits fiber type- and muscle-specific pattern of expression in postnatal rat muscle. Am J Physiol Regul Integr Comp Physiol 278(5):R1381–R1384

Watt DJ, Morgan JE, Clifford MA, Partridge TA (1987) The movement of muscle precursor cells between adjacent regenerating muscles in the mouse. Anat Embryol (Berl) 175(4):527–536

Wokke JH, Van den Oord CJ, Leppink GJ, Jennekens FG (1989) Perisynaptic satellite cells in human external intercostal muscle: a quantitative and qualitative study. Anat Rec 223(2):174–180

Wozniak AC, Anderson JE (2007) Nitric oxide-dependence of satellite stem cell activation and quiescence on normal skeletal muscle fibers. Dev Dyn 236(1):240–250

Yerushalmi GM, Leibowitz-Amit R, Shaharabany M, Tsarfaty I (2002) Met-HGF/SF signal transduction induces mimp, a novel mitochondrial carrier homologue, which leads to mitochondrial depolarization. Neoplasia 4(6):510–522

Ying W (2008) NAD+/NADH and NADP+/NADPH in cellular functions and cell death: regulation and biological consequences. Antioxid Redox Signal 10(2):179–206

Zammit PS, Golding JP, Nagata Y, Hudon V, Partridge TA, Beauchamp JR (2004) Muscle satellite cells adopt divergent fates: a mechanism for self-renewal? J Cell Biol 166(3):347–357

Index

A
Abaxial, 4
Activated satellite cells, 170
Adaxial cells, 52, 54
Adult myogenesis, 49
Ageing, 205, 218
Agnathan-gnathostome divide, 31
Alx4, 145
Ambystoma mexicanum, 150, 154
Amniotes, 29
Amphioxus, 32
Amyotrophic lateral sclerosis (ALS), 178
Angioblasts, 106
AntagomiRs, 182
Anterior digastric, 127
Anterior heart field, 129, 147
Anterior LPM, 147
Aortic arch anomalies, 131
Asymmetric cell division, 104
Autophagy, 225
Avian embryo, 115

B
Basic-helix-loop-helix (bHLH), 196
Becker muscular dystrophy (BMD), 64, 171
Bethlem myopathy, 65
Bipotential stem cells, 203
BMP4, 34, 107, 220
Boc, 90
Bombina orientalis, 154
Bone marrow derived stem cells, 202
Bone morphogenetic protein (BMP), 83
Brachial muscles, 5
Brain-derived neurotrophic factor (BDNF), 198
Branchial arches, 7
Branchial muscle progenitors, 152
Branchiomeric muscles, 7
Branchiomeric muscle satellite cells, 156
Braveheart, 183
Buccinator, 148

C
Cadaver bone marrow, 221
Cadaver satellite cells, 221
Cadaver stem cells, 220
Caenorhabditis elegans (*C. elegans*), 166
Calcitonin, 228
Cancer, 103, 104
Capsulin, 134, 152
Cardiac defects, 131
Cardiac markers, 131
Cardiac progenitor cells, 129
Cardiogenic, 129
Cardiopharyngeal, 133
Cardiotoxin, 171
Cardiovascular progenitors, 131
Casper mutant, 50
β-Catenin, 82, 104, 111, 112, 198
CD34, 200, 220
CD56, 200
CD45+ fraction, 203
Cdo, 90
Cell imaging, 99
Centronuclear myopathy (CNM), 171, 176
Cervical lateral plate, 148
Cervical vertebra, 154
Charcot-Marie-Tooth peripheral neuropathy (CMT), 177

© Springer-Verlag Berlin Heidelberg 2015

B. Brand-Saberi (ed.), *Vertebrate Myogenesis*, Results and Problems in Cell Differentiation 56, DOI 10.1007/978-3-662-44608-9

Chicken–quail transplantation, 115
Chondrichthyans, 26–27
Chordata, 25, 132
Chromatin, 216
Ciona intestinalis, 132
Classical EMT, 103
Clustered regularly interspaced short palindromic repeats (CRISPRs), 66
c-Met, 9, 41, 56, 57
Col6a1, 65
Confocal video microscopy, 99
Congenital fiber type disproportion myopathy, 176
Congenital muscular dystrophies (CMDs), 171
Congenital myopathies, 63, 171, 176
Connective tissue, 32
Connexin 43, 168
Cranial mesoderm, 126
Cranial myogenesis, 152
Cranial neural crest, 134, 154
Cranial paraxial mesoderm (CPM), 144, 149
Cristae, 224
Cucullaris, 148
Cucullaris muscle, 8
CXCR4-inhibitors, 13, 36, 38
CXCR4/SDF1, 107
Cyp26c1, 145
Cytoskeleton, 64

D

Dek, 170
Delta like-1, 197
Depletion of satellite cells, 205
Dermatomyositis (DM), 171, 178
Dermis, 109
Dermo-1, 110
Dermogenic progenitors, 110–111
Dermomyotome, 3
Desmin, 78
Dicer, 166
Digastric, 148
DiGeorge syndrome, 130–131
DiI, 80, 101
DiO, 101
Diphtheria toxin, 204
Dll4, 87
DNA polymerase alpha (Pola1), 168
Dormancy, 229
Dormant state, 225
Dorsomedial lip (DML), 78, 82, 101
Drosha, 165–166
Drosophila, founder cells, 81
Duchenne muscular dystrophy (DMD), 50, 64, 171, 172, 204
Dynamin 2, 177
Dysferlin, 64
Dystrophin, 172, 204
Dystrophin-associated glycoprotein complex (DAPC), 172
Dystrophin gene, 64

E

Electron microscopy, 216
Electroporation, 15
Emery-Dreifuss muscular dystrophy (EDMD), 171
En-1, 111
Endothelial lineage, 107
Endothelial progenitors, 86
Endothelium, 86
Endurance training, 169
Engrailed, 29
Epaxial, 3
Epimysium, 222
Epithelial-to-mesenchymal transition (EMT), 52, 112
ErbB3, 92
ERK/Snail1, 11, 111
Esam, 220
ES cells, 224
Extensor, 34
External cell layer, 53
External eye muscles, 7
Extracellular matrix (ECM), 63, 64
Extraocular muscles (EOM), 125, 126, 149
Eya, 34

F

Facial muscles, 126
Facial subcutaneous muscles, 148
F-actin, 112
Fascioscapulohumeral muscular dystrophy (FSHD), 171
Fast muscle fibres, 217
Fast-twitch, 54
Fgf4/8, 36, 55
FGFR4/FREK, 112
Fibrosis, 174
Flexor muscles, 34
Flk-1/KDR/Quek1, 106
Fmi (Flamingo), 79–80
Follistatin, 228–229
Follistatin-like 1 (Fstl1), 168

Forskolin, 205
Foxc2, 88
FoxF1, 145
Foxo1, 228
Freeze injury model, 221
Frizzled 7, 198

G
Gab1-CXCR4 double-mutant, 13
Gas1, 90
Gastroschisis, 43
Gli1/2, 90, 220
Gli3, 90
Glycolysis, 224
Gremlin, 134
G0 state, 194

H
Hairy, 100
Hand1, 145
Hand2, 145
Head–heart region, 130
Head satellite cells, 129
Heart muscle, 8
Hedgehog-interacting protein (Hhip), 90
Hematopoietic stem cells (HSC), 221
Hepatocyte growth factor (HGF), 222
Hepatocyte growth factor/scatter factor, 198
Hes1, 100
Hes7, 100
Heterogeneity, 200, 201
HIF-1, 224
Histone deacetylase 4 (HDAC4), 167
Homeodomain transcription factors, 32–33
Horizontal myoseptum, 29, 54
Hox code, 33
Human muscle satellite cells, 200
Hypaxial, 3
Hypaxial muscles, 43
Hyperplasia, 58, 59
Hypertrophic cardiomyopathy, 66
Hypertrophy, 58, 59, 199
Hypobranchial muscles, 41, 125
Hypopharyngeal muscle, 41
Hypoxic, 224

I
Immortal strand hypothesis, 201
Inflammatory myopathies, 171, 178
In–Out mechanism, 6
In ovo electroporation, 108
Insulin-like growth factors (IGFs), 199
α7–Integrin, 200
Intracellular domain of Notch (NICD), 88, 197
Irx3, 145
Ischemic necrosis, 221
Isl1, 7, 128, 134, 152
Isl1$^+$ cells, 132
Islet1, 131

J
JAK/STAT pathway, 11

L
Lama2 candyfloss, 65
Laminin a2 (LAMA2), 65
Lamininb2, 65
Lamprey, 31
Late differentiation, 151
Lateral fast fibres, 55
Lateral plate mesoderm (LPM), 144
Lateral plate myogenicity, 158
Lateral somitic frontier, 32
Lateral splanchnic mesoderm, 129
Latissimus dorsi, 5
Lbx1, 9, 34–35, 38, 41
Lbx2, 40
Left ventricle, 129
LGMD. *See* Limb-Girdle muscular dystrophies (LGMD)
LGN, 85, 111
Lhx2, 127
Limb-Girdle muscular dystrophies (LGMD), 64, 65, 171
Limbless, 6
Limb musculature, 8
Limbs, 3
Linc-MD1, 183
Lineage segregation, 85
Lineage tracing, 80
Live confocal video-microscopy, 108
LNA-antimiR, 182
Long noncoding RNAs (lncRNAs), 183
Lou Gehrig's disease, 178
Low-oxygen environment, 225
Lunatic fringe, 100
Lymphatic vessels, 106

M
Malat1, 183
Masseter, 127, 149
Masseter derived satellite cells, 157
m-Cadherin, 200
M. cucullaris, 149

MDC1A, 65
Mdx mice, 64, 172, 204
Medial fast fibres, 55
Mef2c-AHF-Cre transgene, 148
Meox2, 220
Mesoangioblast, 204
MesP1^{+}, 128
MesP^{+} cells, 132
Met, 2
Metabolic rate, 224
MET proto-oncogene, 181
Met tyrosine-kinase receptor, 181
MicroRNAs (miRNA), 83
miR-1, 83, 167
miR-21, 174
miR-23, 169
miR-26, 171
miR-29, 174
miR-31, 170
miR-155, 171, 179
miR-199, 175
miR-206, 83, 167, 170
miR-214, 171
miR-221, 171
miR-222, 171
miR-335, 175
miR-486, 175
miR-489, 170
miR-499, 167
miR-696, 169
miR-133a, 167
miR-133b, 167
miR-146b, 171
miR-208b, 167
miRNA mimics, 182
Mitochondria, 224
Mitotic spindle, 85
Mononucleated myogenic cells, 218
Morphogenetic field, 130
Mrf4, 52, 56, 57, 103, 124
mRNP granules, 170
Msc (MyoR), 7, 127
Msx1, 110
Muscle-less limbs, 32
Muscles
  of facial expression, 125
  hypertrophy, 168
  of mastication, 125
  pioneers, 29, 78
  progenitors, 133
  regeneration, 61, 196
Muscular degenerative diseases, 229
Muscular dystrophies, 63, 169
Musculin/MyoR, 220
Mutant, 13
Myf5, 52, 56, 57, 103, 124
Myf6, 52
Myf5;MyoD, 126
Myf5^{nlacZ}, 196, 200
Myf5 null mutants, 196
Myh6, 168
Myh7, 168
Myh7b, 168
Mylohyoid, 127, 148
Myocardial progenitor cells, 147
Myocardium, 130
Myocyte enhancer factor-2 (MEF2), 167
Myocyte enhancer factor-2C (MEF-2C), 148
MyoD, 52, 56, 57, 103, 124
Myogenic regulatory factors (MRFs), 56, 103, 123, 124, 196
Myogenin (Myog), 52, 56, 57, 103, 124
Myomeres, 54
MyomiRs, 167
Myopathies, 129
MyoR, 134, 152
Myoshi Myopathy, 64
Myosin heavy-chain genes, 168
Myostatin, 114, 169, 219, 228–229
Myotonic dystrophy (DM), 171
Myotonic dystrophy type 1 (DM1), 175

N

N-Cadherin, 79–80, 111, 112
N-CAM, 220
Nemalin myopathy, 171, 176
Nestin, 200
Neural crest cells, 125, 126
Neuregulin1, 92
NICD. *See* Intracellular domain of Notch (NICD)
Nitric oxide (NO), 198
Nkx2.5, 131
Noggin, 134
Notch, 34, 104–105, 107, 199
Notch3, 220
Notch signalling, 197, 206
Numb, 85, 104, 201

O

Osteichthyans, 26–27
Outflow tract, 129
Oxidative phosphorylation, 224
Oxidative stress, 220

P

p21, 219–220
p27, 78
p38, 11
p57, 220
Paraxis, 82, 111
Partner-of-Insc (Pins), 85
Patched1 (Ptc1), 89
Pax3, 8, 56, 124
Pax7, 8, 56, 124, 195, 226
Pax3^{Cre}:Rosa$^{STOP/YFP}$, 150
Pax3/7-FOXO1 fusion genes, 181
Pax genes, 226
Pax3;Myf5, 126
Pax7-nGFP, 200
PDGF-BB, 87
Pectoral fins, 34
Pectoral girdle, 4
Pectoralis major, 5
Pericytes, 88, 204
Perineal/cloacal muscles, 1, 4, 6, 12, 13, 15
Perinuclear mitochondrial arrangement, 224
Perpendicular mitoses, 85
Persistent cloaca, 6
PGC-1α, 169
Pharyngeal arches, 125, 126
Pharyngeal LPM, 147
Pharyngeal mesoderm, 126
Pioneer cells, 102
Pituitary homeobox 2 (Pitx2), 7, 127, 134, 152
Pitx2 mutants, 127
Planar cell polarity (PCP) pathway, 109, 198
Planar orientation, 85
Plasticity of satellite cells, 158
Pluripotency, 223
Pluripotent progenitors, 107
Poland syndrome, 6
Polymyositis (PM), 171, 178
Post-mitotic myocytes, 53
Post-mortem, 221
Post-mortem satellite cells, 225
PPARβ/δ, 169
Prechordal mesoderm, 7, 144
Pre-muscle mass, 6
Premyogenic progenitors, 3
Primary heart field, 147
Primaxial, 3
Pseudostratified columnar epithelium, 100
Pterygoid, 127
Purß, 168
PW1$^+$ cells, 204

Q

Quail-chick cell lineage tracing, 153
Quail–chick chimaeras, 156, 193
Quantal mitosis, 218
Quiescence, 194
Quiescent stem cells, 170

R

Rapamycin (mTOR), 224–225
Rbpj, 197
Reactive oxygen species (ROS), 224
Red muscle, 59
Regenerative medicine, 229
"Reserve cells," 198
Resistance training, 169
Reverse EMT, 103
Rhabdomyosarcomas (RMS), 11, 179
RhoA, 80, 81
Rhomboidei, 5
Right ventricle, 129
RNA-induced silencing complex (RISC), 166
Robo2, 80, 81
Ryanodine receptor1 (RyR1), 65

S

Sapje (sap^{ta222a}), 64
Sarcopenia, 50, 205, 206
Satellite cell motility, 222
Satellite cells, 54, 128, 156, 169–170
Scapula blade, 77
Scatter factor/hepatocyte growth factor
(SF/HGF), 2, 9, 36, 41
SDF-1, 2, 10–13, 15–18, 20–22, 242
Secondary heart field, 129–130
Segmentation clock, 100
Self-renewal, 196
Serratus anterior, 5
Serum response factor (SRF), 168
SF/HGF mutants, 38
Shoulder muscles, 5
Side population, 203
Silent heart, 66
Six1, 127
Six transcription factors, 34
Skeletal muscle forming potential, 148
Slit1, 81
Slow cycling stem cell population, 113
Slow muscle fibres, 217
Slow myosin heavy chain, 55

Slow-twitch muscle fibres, 55
Slow-twitch myosin heavy chain, 54
Small noncoding RNAs, 165
SM/C-2.6, 200
Smoothened (Smo), 89
Smooth muscle, 86
Sod genes, 225
Somatopleura, 144
Somatopleure, 145
Somite rotation, 53
Somitic extensions, 34, 41
Sonic hedgehog (Shh), 7, 29, 54, 83, 89
Sox6, 168
Sp3, 168
Splanchnopleura, 144, 145
Splotch, 8, 36
Sporadic inclusion-body myositis (IBM), 178
Sprouty1, 198
Steinert's disease, 175
Stemness, 193
Sternalis muscle, 6
Sternocleidomastoideus, 150
Stress resistance, 220
Stromal-cell-derived factor 1, 10
Styloid, 148
Sulfatase1, 90
Syndecan1/4, 200

T
Targeting induced local lesions in genomes (TILLING), 66
Tbx1, 7, 126, 127, 134, 152
Tbx20, 147
Tbx1$^{-/-}$mouse mutants, 153
Tcf21 (capsulin), 7, 127
Tcf21/Msc double mutants, 127
TDSS, 201
Teleosts, 27
Template DNA strand co-segregation, 201
Temporalis, 127, 149
Texel sheep, 169
Therapeutics, 229
Thymosin beta 15-like peptide, 153
Tissue sheet, 41
Tissue-specific stem cell, 206
TNNC1, 66
TNNT2, 66
Tongue, 3
  and intrinsic laryngeal muscles, 7
  muscles, 125
Transcription activator-like effector nucleases (TALENs), 66
Transit amplification, 198
Transit amplifying population, 113
Translaminal migration, 222
Trapezius, 150
TroponinT, 227
Troponin TNNT3, 66
Troponin/tropomyosin complex, 65
Tunicates, 132
Twist, 145

U
Ulk1, 225
Ullrich congenital Muscular Dystrophy, 65
Unorthodox stem cells, 202
3' Untranslated regions (UTR), 166
Utrophin, 168

V
Vascular endothelial cells, 106
Vascular endothelial growth factor (VEGF), 198
Vascular endothelial growth factor receptor 2 (VEGFR2), 106, 107
VCAM-1, 220
Vegfr2, 89
Ventrolateral dermomyotome, 8
Ventrolateral lips (VLL), 82, 101
Vertical myosepta, 54

W
White muscle, 59
Wnt, 7, 104
Wnt4, 198
Wnt6, 111, 198
Wnt11, 83, 109, 110
Wnt7a, 198
Wnt signaling, 82, 198

X
Xenobiotics, 220

Z
Zinc finger nucleases (ZFN), 66