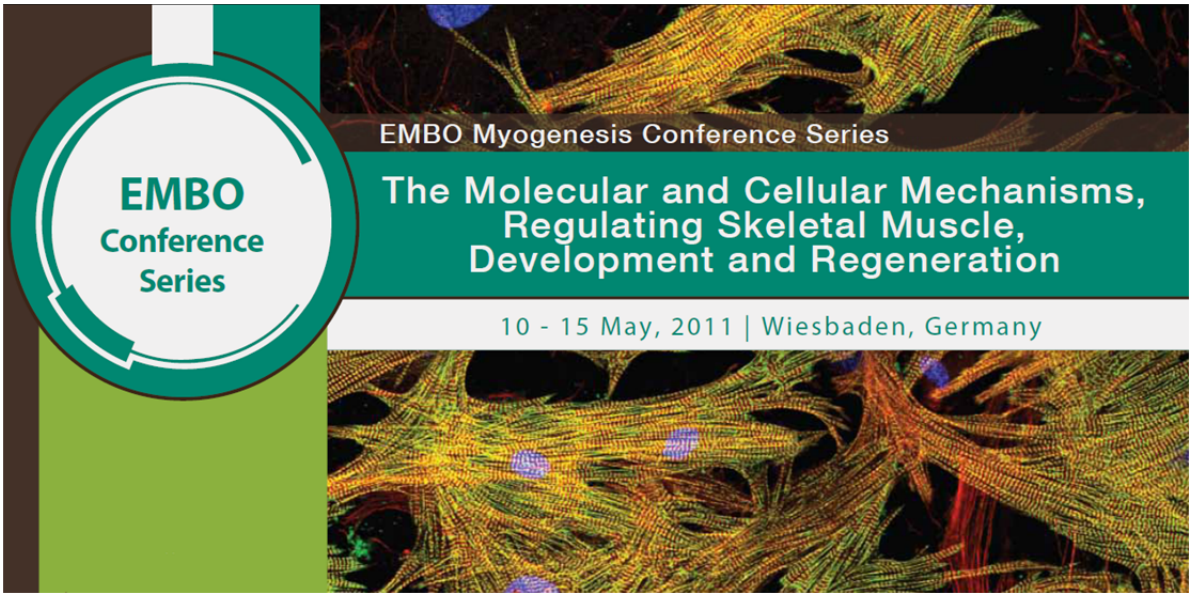

DORINT PALLAS HOTEL, Wiesbaden, Germany

10th-15th May 2011

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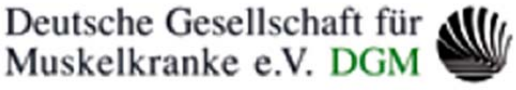

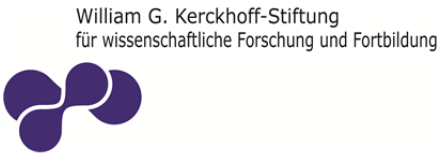

LOCAL ORGANISERS

SUSANNE SCHNEIDER
SUSANNE MARTIN
MATTHIAS HEIL

CONTENT

PROGRAM

Tuesday, 10th May 2011

2.00 - 6.00 pm **Arrival and Registration**
6.00 pm **Opening of Conference**

6.15 pm **Keynote Lecture**
Hans R. Schöler – *Induction of pluripotency in adult stem cells*

7.00 pm Buffett

Evening Session – Model Organisms
8.00 pm – 9.30 pm
Chair: Philip W. Ingham
8.00 pm Helen Blau - *Of Newts and Niches: Regenerating tissues by mimicking natural processes*
8.30 pm Frank Schnorrer - *The making of flight muscles*
9.00 pm Alejandro Sánchez Alvarado – *Regeneration, Stem Cells and the Planaria Schmidtea mediterranea*

Wednesday, 11th May 2011

Somite Patterning and Muscle Development I
9.00 am – 12.10 pm
Chair: Thomas Braun
9.00 am Christophe Marcelle – *Neural Crest Regulates Myogenesis through the Transient Activation of NOTCH*
9.30 am Eldad Tzahor – *Redefining the transcriptional network of pharyngeal mesoderm reveals a novel pathway for the DiGeorge syndrome*
10.00 am *Delphine Duprez - Intrinsic regulators of muscle progenitors during embryonic myogenesis*
10.15 am *Coffee break*
11.00 am Simon Hughes - *Muscle formation and growth in the zebrafish*
11.30 am Krzysztof Jagla – *Genetic control of muscle-type specific differentiation programs: insights from Drosophila*
11.55 am Laurence Dubois - *A genetic screen to identify new genes involved in specification of muscle identity in Drosophila: interaction between tailup (tup) and collier (col) specify DA2 and DA3 muscle identity.*

12.10 pm *Lunch*

Wednesday, 11th May 2011, cont.

Somite Patterning and Muscle Development II
1.15 pm – 4.30 pm
Chair: Frank Stockdale

1.15 pm Chaya Kalcheim - *LGN-dependent orientation of cell divisions in the dermomyotome controls lineage segregation into muscle and dermis*
1.45 pm Philip Ingham – *Cell type specification in the zebrafish myotome: signals, transcription factors and miRNAs*
2.15 pm Robert Kelly - *Myogenic derivatives of pharyngeal mesoderm*
2.45 pm Chrissa Kioussi - *Motility of Myogenic Cells in Filling Limb Muscle Anlagen*
3.00 pm *Coffee break*
3.30 pm Pete Currie – *Development and Growth of the Zebrafish myotome*
4.00 pm Huai-Jen Tsai – *Novel microRNA-In300 and its target gene dickkopf-3-related gene regulate the promoter activity of Muscle Regulatory Factor myf5 gene*

Genetic Control of Muscle Cell Differentiation
4.30 pm – 7.30 pm
Chair: Pura Munoz-Canoves

4.30 pm Frederic Relaix – *Molecular mechanisms coordinating myogenesis and cell cycle regulation during development*
5.00 pm Robert S. Krauss – *Regulation of promyogenic signal transduction by cell-cell contact and adhesion*

5.30 pm *Coffee break*

6.00 pm Hans-Henning Arnold - *Canonical Wnt signaling in early and late mouse myogenesis*
6.30 pm Jaime Carvajal - *Regulation of MRF expression in facial muscle precursors*

7.00 pm George Muscat - *Regulation of skeletal muscle insulin signaling by the nuclear receptor, RORalpha.*
7.30 pm Stephen D. Hauschka - *Basic regulatory controls of muscle gene expression*

8.00 pm **Dinner**

Poster Session
9.00 pm- 10.30 pm

Thursday, 12th May 2011

Posttranscriptional Control of Myogenesis (miRNAs) and Epigenetics
8.30 am – 12.45 pm
Chair: Stephen D. Hauschka

8.30 am Eric Olson – *New Insights into the Genetic Networks of Skeletal Muscle Disease*
9.00 am Carola Ponzetto - *MicroRNAs in myogenesis: from physiology to pathology and back*
9.30 am Andrea Münsterberg – *MicroRNAs confer robustness to developmental timing of myogenesis*
10.00 am Irene Bozzoni - *Epigenetic control of miRNA expression through the dystrophin-nNOS signalling cascade*

10.25 am *Coffee break*

11.00 pm **Keynote Lecture**
Howard Cedar - Reprograming

11.45 am Margaret Buckingham – *The modulation of myogenic behaviour in progenitor cells*
12.15 am Pier Lorenzo Puri – *Epigenetic regulation of muscle regeneration by HDAC-regulated miRNA-SWI/SNF circuitry*
12.45 pm Davide Gabellini - *A chromatin-associated RNA regulates a Polycomb/Trithorax epigenetic switch at the basis of facioscapulohumeral muscular dystrophy (FSHD)*
1.00 pm Bruce Paterson – *Drosophila nautilus (MyoD) activates a miRNA circuit that fine-tunes dMef2, tinman and output from the Notch and Jak-Stat pathways to regulate somatic muscle formation*

1.15 pm *Lunch*

Epigenetic Regulation and Reprograming
02.15 pm – 4.15 pm
Chair: Michael Rudnicki

02.15 pm Vittorio Satorelli – *Epigenetics of Skeletal Myogenesis.*
02.45 pm Anthony Imbalzano - *Spatial re-organization of gene regulatory sequences temporally controls myogenic gene expression*
03.15 pm Coffee break

03.30 pm Thomas Rando - *Molecular regulation of muscle stem cell quiescence*
04.00 pm Atsushi Asakura - *MyoD gene suppression by Oct4 is required for reprogramming in myoblasts to produce induced pluripotent stem (iPS) cells*

04.30 pm **Excursion to "Rheingau" incl. Dinner**
Bustransfer to world famous Rüdesheim
Boarding on Cruiseship Loreley-Elegance
4 h Diner Rhine-River-Shuffle

Friday, 13 May 2011

Satellite Cells and Stem Cell Properties
8.30 am – 12.15 pm
Chair: Margaret Buckingham

8.30 am Chen-Ming Fan - *The Pax7 expressing lineage*
9.00 am Michael Rudnicki - *Molecular Regulation of Muscle Stem Cell Function*
9.30 am Peter Zammit - *The regulation of muscle satellite cell fate choice*
10.00 am Athanassia Sotiropoulos - *Srf-dependent paracrine signals produced by myofibers control satellite cell-mediated skeletal muscle hypertrophy*

10.15 am *Coffee break*

11.00 am Johnny Kim - *Identification of epigenetic modifiers involved in muscle stem cell differentiation*
11.15 am David Sassoon - *p53 effectors, muscle stem cells, and aging: towards a common nodal point in adult stem cell regulation*
11.45 am Bradley Olwin – *The Role of the Satellite Cell Niche in Cell Homeostasis and Aging*

12.15 pm *Lunch*

Cell Signaling and the Stem Cell Niche
1.15 pm – 4.10 pm
Chair: Helen Blau

1.15 pm Carmen Birchmeier – *N.N.*
1.45 pm Shahragim Tajbakhsh – *Satellite cell properties and function*
2.15 pm Ketan Patel – *Regulation of Satellite Cell Migration*

2.40 pm *Coffee break*

2.55 pm Fabio Rossi - *Regulation of the balance between repair and regeneration in skeletal muscle*
3.25 pm Andrew Brack - *Regulation of satellite cell quiescence during muscle growth*
3.55 pm Sören Alsheimer - *Muscle Stem Cells Secrete Tenascin W, a Key Component of Their Niche*
4.10 pm *Coffee break*

Systems Biology
4.40 pm – 5.40 pm
Chair: Carmen Birchmeier

4.40 pm Eileen Furlong – *Linking transcription factor occupancy and chromatin state to gene expression during embryonic development*
5.10 pm Abraham Fong – *Comparison of the Myogenic and Neurogenic Regulatory Programs*

Therapeutic Approaches to Muscle Diseases
5.40 pm – 7.10 pm
Chair: Christophe Marcelle

5.40 pm Giulio Cossu - *A novel strategy for autologous cell therapy for Duchenne Muscular Dystrophy*
6.10 pm Gert-Jan von Ommen – *Antisense-based exon skipping as a therapy for Duchenne Muscular Dystrophy, status and prospects*

7.30 pm **Dinner**

Poster Session
9.00 pm- 10.30 pm

Saturday, 14 May 2011

Organ Development I (ECM, Nerve-Muscle Interactions, Migration, Myoblast Fusion)
8.30 am – 10.45 pm
Chair: Krystzof Jagla

8.30 am Steve Burden - *Muscle-derived Signals For Regulating Motor Innervation*
9.00 am Benny Shilo – *Regulation of muscle cell fusion by actin nucleation*
9.30 am Renate Renkawitz-Pohl - *DSwiprosin-1/EFhd2 accumulates in FuRMAS at the prefusion complex stage during Drosophila myoblast fusion*
10.00 am Mary Baylies - *MAP and Kinesin dependent nuclear positioning is required for skeletal muscle function*
10.30 am Jeffrey Dilworth - *Comparative gene expression profiling identifies the tetraspanin CD53 as a p38alpha-dependent protein that modulates cell fusion*

10.45 am *Coffee break*

Organ Development II
11.30pm – 1.15 pm
Chair: Renate Renkawitz-Pohl

11.30 am Olivier Pourquie – *Early patterning and differentiation of vertebrate muscles*
12.00 pm Laurent Schaeffer – *Neural control of muscle gene expression: CtBP mediates repression*
12.30 pm Ilona Skerjanc - *Skeletal myosin light chain kinase regulates skeletal myogenesis by phosphorylation of MEF2C*
12.45 pm Malcom Logan - *Regulation of limb muscle morphogenesis*

01.15 pm *Lunch*

Regenerative Biology
2.15 pm – 4.00 pm
Chair: Peter Rigby

2.15 pm Andras Simon - *Dynamics of skeletal muscle cells during newt limb regeneration*
2.45 pm Elly Tanaka - *Reformation of Muscle and Skeleton during Axolotl Limb Regeneration*
3.15 pm Nadia Rosenthal - *Immune modulation of muscle regeneration*
3.45 pm Rachel Sarig - p53 plays a pivotal role in preventing malignant transformation of induced pluripotent stem cells
4.00 pm *Coffee break*

4.45 pm Stefano Schiaffino - *Novel players in the regulation of muscle growth*
5.15 pm David J. Glass - *Signaling pathways that mediate skeletal muscle atrophy and hypertrophy*
5.45 pm Alfred Goldberg - *New Insights into the Molecular Mechanisms of Muscle Atrophy*

6.15 pm **Excursion to the Cistercian Eberbach Monastery incl. Wine-Tasting and Farewell Dinner**
Known from the famous movie "The Name of the rose"

Sunday, 15 May 2011

Breakfast and Departure
8.30 am – 12.00 pm

ABSTRACTS - ORAL PRESENTATIONS

(sorted by program)

Of Newts and Niches: Regenerating tissues by mimicking natural processes

Helen Blau
Stanford University School of Medicine

Baxter Laboratory for Stem Cell Biology, Stanford University School of Medicine, Stanford University, CA

"Of Newts and Niches: Regenerating tissues by mimicking natural processes"
We are exploiting natural mechanisms to derive mammalian cell sources for regenerative medicine: (1) by recapitulating pathways used by newts and zebrafish, (2) by mimicking biophysical cues to which adult stem cells are exposed in the body, (3) by gaining insights into the mechanisms such as DNA demethylation by which adult cells are reprogrammed to pluripotency (iPS) by cell fusion in heterokaryons and (4) by studying stem-cell based diseases such as Duchenne Muscular Dystrophy. These approaches provide fundamental mechanistic insights into stem cell fate determination and should enable clinical applications.

Notes

The making of flight muscles

Cornelia Schönbauer , Manuela Weitkunat , Aynur Kaya-Copur , Frank Schnorrer
Max-Planck-Institute of Biochemistry, Am Klopferspitz 18, 82152 Martinsried, Germany

Muscle is a powerful biological machine enabling controlled movements of higher animals. The Drosophila adult musculature is built from stem cell like adult myoblasts in pupae that fuse with each other, target to their proper tendons and assemble highly organised myofibrils in a muscle-type specific manner, e.g. fast moving, fibrillar flight muscles powering wing oscillations or striated, tubular leg muscles that enable walking. We image the entire process of adult myogenesis in living pupae from myoblast fusion until sarcomere formation and identify key regulators of the different steps by a systematic RNAi based strategies. Thus, we found that the transmembrane protein Kon-tiki (Kon) is required in the migrating myotubes for targeting to the proper tendons. In kon knock-down pupa a flight muscle - tendon connection fails to be established. We identified the transcription factor spalt major (salm) as a master regulator of fibrillar flight muscle. After myoblast fusion salm expression is specifically induced in the developing flight muscles by vestigial. salm is not only required but also sufficient to induce the fibrillar muscle fate when expressed ectopically. Hence, salm can overrule other muscle identity programs. It is responsible for all features characteristic to fibrillar flight muscles; in particular, it regulates the expression and splicing of various sarcomeric proteins that execute the fibrillar muscle fate. We find that this function of spalt is conserved in insects separated by 120 million years of evolution and therefore appears to constitute an ancient developmental principle.

Notes

Neural Crest Regulates Myogenesis through the Transient Activation of NOTCH

Christophe Marcelle, Anne Rios, Olivier Serralbo, David Salgado

The early skeletal muscle (the primary myotome, composed of mononucleated, post-mitotic muscle fibers, the myocytes) is formed from the generation of muscle cells at the four borders of the dermomyotome, the dorsal-most epithelial compartment of somites. Most of the dermomyotome undergoes an epithelial to mesenchymal transition that leads to the emergence of a population of resident muscle progenitors that massively contributes to the growth of all trunk muscles. The medial border of the dermomyotome (DML) remains epithelial for a considerable period of time, during which it generates muscle cells that contribute to the growth of the primary myotome. DML stem/progenitor cells can adopt two fates during the first days of embryonic muscle development: to self-renew and remain in the epithelial border of the dermomyotome or to translocate in the myotome and undergo terminal myogenic differentiation. How this balance is regulated is unknown. We show that muscle progenitors present in the DML require the transient activation of NOTCH signaling to undergo terminal differentiation. The NOTCH ligand Delta1 is expressed in a mosaic pattern in neural crest cells that migrate past the somites. Gain and loss of Delta1 function in neural crest modifies NOTCH signaling in somites, which results in delayed or premature myogenesis. Our results suggest that the neural crest regulates early muscle formation by a unique mechanism that relies on the migration of Delta1-expressing neural crest cells to trigger the transient activation of NOTCH signaling in selected muscle progenitors. This dynamic signaling guarantees a balanced and progressive differentiation of the muscle progenitor pool.

Notes

Redefining the transcriptional network of pharyngeal mesoderm reveals a novel pathway for the DiGeorge syndrome

Itamar Harel [1], Yoshiro Maezawa [2], Hadar Hay [3], Achira Roy [4], Julius Hegesh [5], Roi Avraham [1], Ariel Rinon [1], Shubha Tole [4], Sue Quaggin [2], Eldad Tzahor [1]

[1] 1. Department of Biological Regulation, The Weizmann Institute of Science, Rehovot 76100, Israel.

[2] 2. The Samuel Lunenfeld Research Institute, TCP Building, Room 5-1015-2, 25 Orde Street, Toronto, Ontario M5T 3H7, Canada.

[3] 1. Department of Biological Regulation, The Weizmann Institute of Science, Rehovot 76100, Israel

[4] 5. Department of Biological Sciences, Tata Institute of Fundamental Research, Mumbai 400 005, India

[5] 3. Department of Pediatric cardiology, The Chaim Sheba Medical Center and Sackler School of Medicine, Tel Aviv University, Tel Aviv 69978, Israel.

Pharyngeal mesoderm (PM) progenitors are located in the head region of the developing embryo, contributing to broad regions of the heart, as well as to the head musculature. PM progenitors express a set of transcription factors, distinct from that of trunk muscle progenitors. It is not clear how these factors interact in a hierarchical regulatory network coregulating myogenesis and cardiogenesis. In order to identify novel PM regulators in the mouse, we performed a gene expression array from isolated PM progenitors at early stages of head myogenesis (E9.5-E11.5). In addition to known regulators (e.g, Tbx1, Pitx2, Tcf21 and Islet1), we identified a novel regulator in this network, Lhx2, a LIM-domain containing transcription factor. Lhx2 knockout embryos exhibited reduced PM myogenic progenitors, as well as broad muscle patterning defects. Genetic ablation experiments revealed hierarchical cross regulation within members of the PM network, which may serve to fine-tune and stabilize the network outputs. A key gene in this network is Tbx1; its absence in humans can lead to the DiGeorge syndrome. Epistatic genetic analysis demonstrated that Tcf21 regulates Tbx1, which is upstream to Lhx2. Genetic ablation of each of these factors perturbed PM development, leading to congenital heart and craniofacial muscle defects, phenocopying the DiGeorge syndrome. Our findings reveal a regulatory circuit in PM progenitors, which is essential for cardiac and craniofacial development.

Notes

Intrinsic regulators of muscle progenitors during embryonic myogenesis

Emmanuelle HAVIS [1], *Pascal COUMAILLAU* [2], *Jacques MICHAUD* [3], *Delphine DUPREZ* [2]
[1] *UPMC-CNRS, UMR7622, Laboratoire de Biologie du Développement, paris, France*
[2] *UPMC-CNRS, UMR7622, Laboratoire de Biologie du Développement*
[3] *Centre de Recherche du CHU Sainte-Justine, Canada*

Skeletal muscle development, growth and regeneration rely on muscle stem cells. An important goal is to understand the source and the nature of the signals regulating these muscle stem cells during myogenesis. Intrinsic and extrinsic regulatory pathways control the pool of muscle progenitors at different developmental times and places, during embryonic, foetal and peri-natal myogenesis. As an intrinsic signal, we have identified the bHLH-PAS, Sim2 (Single minded-2) gene as a regulator of embryonic muscle progenitors. Sim2 expression is mutually exclusive with that of MyoD. The regulation of Sim2 expression by Notch signalling is similar to that of muscle progenitor markers. Sim2 gain- and loss-function experiments indicate that Sim2 represses MyoD expression. Moreover, we show that Sim2 represses the activity of regulatory elements of the MyoD promoter. Lastly, Sim2 is recruited to regulatory elements of the MyoD promoter, in vivo. In summary, Sim2 regulates embryonic muscle progenitors by repressing MyoD expression.

Notes

A genetic screen to identify new genes involved in specification of muscle identity in Drosophila: interaction between tailup (tup) and collier (col) specify DA2 and DA3 muscle identity.

Laurence Dubois [1], Hadi Boukhatmi [1], Jonathan Enriquez [2], Jean louis Frendo [1], Michèle Crozatier [1], Alain Vincent [1]
[1] Centre de Biologie du Développement, UMR 5547 et IFR 109, CNRS, 118 route de Narbonne, Toulouse, France.
[2] Department of Biochemistry and Molecular Biophysics, Columbia University, 701 W. 168th St., HHSC 1104 New York, New York 10032 USA

What are the molecular and cellular mechanisms controlling muscle identity (e.g., shape, orientation, size)? Our laboratory uses the fruit fly, Drosophila melanogaster, as a model organism to study this question. The Drosophila larval musculature displays a stereotyped pattern of about 30 muscles per hemisegment, each one being made of a single multinucleated fibre originating from a founder cell. Founder cells originate from asymmetric division of progenitor cells selected from equivalence groups of myoblasts. The traditional view is that the properties specific to each muscle reflect the combination of 'identity' transcription factors (iTFs) expressed by its founder cell. Expression of the iTF Collier (Col), the Drosophila ortholog of mammalian Early-B Cell Factors (EBFs), is activated in one dorso-lateral equivalence group, from which at least three progenitors are selected. Recent analysis has shown a temporal sequence of emergence of these "Col-expressing progenitors", each of them expressing different iTFs. col mutant embryos shows muscle transformations in dorso-lateral muscles, such as a DA3>DA2 transformation (DA stands for Dorsal Acute muscle), indicative of a shift in the identity of the DA3 progenitor. From a genetic screen designed to identifying new genes involved in the specification of dorso-lateral muscle progenitors, we identified tailup (tup) the Drosophila ortholog of vertebrate islet 1, whose loss of function induces the reciprocal DA2>DA3 transformation. We will describe how interactions between col and tailup control the distinction and specification of DA3 and DA2 muscle progenitor identity.

Notes

LGN-dependent orientation of cell divisions in the dermomyotome controls lineage segregation into muscle and dermis

Raz Ben-Yair, Nitza Kahane, Chaya Kalcheim

The plane of cell divisions is pivotal for differential fate acquisition. Dermomyotome development provides an excellent system to investigate the link between these processes because single cells in the early dermomyotome divide with a planar orientation and either self-renew or generate myofibers, whereas in the late epithelium they divide perpendicularly to produce both mitotic myoblasts and dermis. To examine whether spindle orientations influence fate segregation, early planar divisions were randomized and/or shifted into perpendicular by interfering with cLGN or by overexpressing inscuteable. Clones derived from single transfected cells exhibited an enhanced proportion of mixed dermomyotome/myotome progeny at the expense of like-daughter cells. Loss of LGN or Gαi function in the late epithelium randomized otherwise perpendicular mitoses and favored muscle development at the expense of dermis. Hence, LGN-dependent early planar divisions are required for proper allocation of progenitors into either dermomyotome or myotome while late perpendicular divisions are necessary for the normal balance between muscle and dermis production.

Notes

Myogenic derivatives of pharyngeal mesoderm

Robert Kelly
Developmental Biology Institute of Marseilles-Luminy

Mesoderm associated with the pharynx or foregut in the early embryo has two striated muscle derivatives: cardiac muscle and branchiomeric craniofacial skeletal muscles. The latter differ from all other muscles in the embryo and include muscles of mastication derived from the first branchial arch, muscles of facial expression from the second arch and laryngeal and pharyngeal muscles. Branchiomeric muscles are derived from a progenitor cell population expressing Tbx1, Isl1 and Fgf10, genes that are also expressed in adjacent cardiac progenitor cells contributing to the right ventricle and outflow tract of the heart. Retrospective lineage experiments have revealed a clonal relationship between branchiomeric muscles and parts of the heart. In particular first arch muscles are clonally related to cells of the right ventricle and second arch muscles to cells of the cardiac outflow tract. This lineage relationship reflects the caudal movement of the heart in the pharyngeal region during heart tube elongation. Tbx1 regulates the robustness of branchiomeric myogenic specification and sporadic formation of first and second arch muscles in Tbx1 mutant embryos is largely dependent on the myogenic regulatory factor Myf5. Tbx1 also regulates cardiac progenitor cell development, in particular the addition of a population of cells to the right ventricular outlet. Tbx1, together with Tbx3, regulates the signalling environment or niche of cardiac progenitor cells during heart tube elongation, controlling the balance between proliferation and differentiation. The divergent myogenic fates of pharyngeal mesoderm and role of Tbx1 illustrate the importance of the cardiocraniofacial mesodermal field in development and pathology.

Notes

Motility of Myogenic Cells in Filling Limb Muscle Anlagen

Chrissa Kioussi
Oregon State University

Cells of the ventrolateral dermomyotome delaminate and migrate into the limb buds where they give rise to all muscles of the limbs. The migratory cells proliferate and form myoblasts, which withdraw from the cell cycle to become terminally differentiated myocytes. The myogenic lineage colonizes pre-patterned regions to form muscle anlagen as muscle fibers are assembled. The regulatory mechanisms that control the later steps of this myogenic program are not well understood. The homeodomain transcription factor Pitx2 is expressed specifically in the muscle lineage from the migration of precursors to adult muscle. Ablation of Pitx2 results in distortion, rather than loss, of limb muscle anlagen, suggesting that its function becomes critical during the colonization of, and/or fiber assembly in, the anlagen. Microarrays were used to identify changes in gene expression in flow-sorted migratory muscle precursors, labeled by Lbx1GFP/+, which resulted from the loss of Pitx2. Very few genes showed changes in expression. Many small-fold, yet significant, changes were observed in genes encoding cytoskeletal and adhesion proteins which play a role in cell motility. Myogenic cells from genetically-tagged mice were cultured and subjected to live cell-tracking analysis using time-lapse imaging. Myogenic cells lacking Pitx2 were smaller, more symmetrical, and had more actin bundling. They also migrated about half of the total distance and velocity. Decreased motility may prevent myogenic cells from filling pre-patterned regions of the limb bud in a timely manner. Altered shape may prevent proper assembly of higher-order fibers within anlagen. Pitx2 therefore appears to regulate muscle anlagen development by appropriately balancing expression of cytoskeletal and adhesion molecules.

Notes

Development and Growth of the Zebrafish myotome

Peter Currie
Australian Regenerative Medicine Institute

Zebrafish adaxial cells are myogenic precursors that form a cell fate equivalence group of approximately 20 cells, initially specified by Hedgehog signals secreted from the embryonic midline. Adaxial cells consequently give rise to two distinct sub-types of muscle fibres: the superficial slow muscle fibres (SSFs) and muscle pioneer cells (MPs), distinguished by specific gene expression and cell behaviours. Within the zebrafish myotome SSFs and MPs, possess distinct morphological, molecular and functional properties. SSFs migrate from the midline to the surface of the myotome and form a monolayer of differentiated slow twitch muscle fibres, while the MPs (2 to 6 per somite) are the first cells of the zebrafish myotome to differentiate, are non migratory and differentiate immediately adjacent to the notochord. It is currently unclear how the equivalence of the adaxial cell group is broken, given that the secreted HH signal is likely to be received by all adaxial cells equally. In order to understand the origins of SSF and MP precursors from within the adaxial cell compartment, we examined adaxial cell behaviours at the somitogenesis and during the first phase of their differentiation via continuous 4D time-lapse analysis and retrospective fate map analysis of the entire forming myotome. We show that distinct fates derive from topgraphically separable progenitors and we define the molecular signals that coordinate the specification of adaxial cell compartment.

Notes

Novel microRNA-In300 and its target gene dickkopf-3-related gene regulate the promoter activity of Muscle Regulatory Factor myf5 gene

Huai-Jen Tsai

Novel microRNA-In300 and its target gene dickkopf-3-related gene regulate the promoter activity of Muscle Regulatory Factor myf5 gene
Ren-Jun Hsu, Cheng-Yung Lin, Chiu-Chun Lin and Huai-Jen Tsai
Institute of Molecular and Cellular Biology, National Taiwan University, Taiwan
Myf5, one of myogenic regulatory factors, plays important roles in the specification and differentiation of muscular cells during myogenesis. The detailed molecular mechanism underlying the negative control of myf5 expression in somite is still unclear. Recently, we found an intronic microRNA (miR) located at +610/+632 within the first intron +502/+835 (In300) of zebrafish myf5, termed miR-In300 or miR-3906, which significantly represses the myf5 promoter activity. Using Labeled miR pull-down assay (LAMP) we developed, we identified that dickkopf-3-related gene (dkk3r) is one of the target genes of miR-In300. Knockdown of dkk3r did result in down-regulation of myf5 in somites, whereas co-injection of myf5 mRNA with dkk3r-morpholino (MO) enabled rescue of the defects induced by the knockdown of dkk3r and the expression of myogenin, a myf5 downstream gene, in somites. Therefore, we concluded that miR-In300 binds to the dkk3r, which, in turn, inhibits the translation of the Dkk3r protein and results in suppressing the expression of myf5, thus establishing miR-In300 as a negative regulator of zebrafish myf5.
Furthermore, we found that the phosphorylated p38a protein in embryos was reduced if dkk3r-MO was introduced. Knockdown of p38a resulted the malformed somites and the reduced myf5 transcripts, which photocopied the defects induced by injection of dkk3r-MO. Blocking the MAPK pathway through interfering the phosphorylation of p38 caused the down-regulation of myf5 expression. The GFP signal was dramatically decreased in somites when we injected p38a-MO into the embryos derived from transgenic line Tg(myf5(80K):GFP), in which the GFP was driven by myf5 promoter. This p38a-MO-induced defects were rescued by co-injection with p38a mRNA, but were not rescued with the mutated p38a mRNA containing a mutation at phosphorylation domain. Moreover, over-expression of Smad2 or Smad 3a enhanced myf5 expression, but the defects induced by the dominant-negative form of either Smad2 or Smad 3a equaled those of embryos injected with either dkk3r-MO or p38a–MO. These results support the involvement of Smad2/3a in p38a modulation. Over-expression of Smad4 enabled the rescue of myf5 defects in the dkk3r-MO-injected embryos, but knockdown of either dkk3r or p38a caused Smad4 protein to lose stability. Therefore, we concluded that Dkk3r regulates p38a phosphorlylation to maintain Smad4 stability, enabling the Smad2/ 3a /4 complex to form and activate myf5 promoter.

Notes

A molecular mechanism coordinating myogenesis and cell cycle regulation during development

Keren Bismuth [1], Ted Chang [1], Zizhen Yao [2], Yi Cao [2], Frederic Aurade [1], Stephen Tapscott [2], Frederic Relaix [1]
[1] UPMC, INSERM UMR S 787- Groupe Myologie, Paris, France
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Muscle cell formation is a coordinated process of tissue-specific gene expression, proliferation and differentiation. In order to safeguard the developing progenitor cells against a prolonged proliferation or a premature differentiation, cell cycle exit has to be tightly regulated. In the myogenic lineage, irreversible cell cycle exit is controlled by the Cyclin Dependent Kinase Inhibitors (CDKIs) p21 and p57. Mice lacking these two genes harbor severe muscle defects.
Using MyoD-/- and p21-/-; p57+/-m mutant embryos, we found that Myf5 is sufficient to trigger p57 expression, indicating that cell cycle exit is associated with cell determination. Moreover we show that during development, growth arrest is uncoupled from cell differentiation. Next, using MyoD-/-; Myf5nLacZ/nLacZ mutant, in which myogenesis is abrogated, we found that determined myoblasts control Pax3+ cells proliferation by preventing them from activating p57 and exiting cell cycle. We show that this control is mediated by the Notch pathway through the Notch-regulated genes Hes1, Hey1 and HeyL, which are down regulated in MyoD-/-; Myf5nLacZ/nLacZ mutant embryos. Finally, we have identified a p57 specific Muscle Regulatory Element and found that, in vivo, bHLH transcription activator MyoD and the transcriptional repressor Hes1 are bound to this element. Thus, we have identified a molecular mechanism whereby the direct interaction between early post-mitotic myoblasts and progenitor cells allows the activation of Notch target gene Hes1 in Pax3+ cells, which in turn suppress p57 expression, keeping the progenitor cell cycling. This mechanism ensures the regulated equilibrium between building muscles and maintaining the pool of undifferentiated progenitors cells.

Notes

Regulation of promyogenic signal transduction by cell-cell contact and adhesion

Robert Krauss , Min Lu
Mount Sinai School of Medicine

The small GTPases Rac1 and Cdc42 are required for mammalian skeletal myogenesis in vivo and in vitro. Rac1 and Cdc42 function as signaling hubs, activated by many extracellular cues and transducing signals via many effector proteins. Paks are S/T kinases that are major effectors of Rac1/Cdc42 and regulate cell polarity, actin dynamics and gene expression. We report Pak1 and Pak2 are expressed in primary mouse myoblasts and C2C12 cells in vitro and are activated specifically during differentiation. Cell-cell contact promotes myogenic differentiation, and N-cadherin (Ncad) ligation activated Pak1 and Pak2 in a manner that required the Ncad-associated Cdo complex, which includes signaling modules for Cdc42 and p38α/β MAPK. Pak1 and Pak2 formed complexes with p38 during differentiation, in a manner dependent on the p38 scaffold JLP. Ncad-initiated p38 activation required Pak2, but not Pak1. Chemical and dominant negative inhibitors of Pak1/2 kinase activity blocked differentiation as assessed by expression of muscle proteins and myotube formation. Depletion of Pak2 by RNAi had a similar inhibitory effect, whereas depletion of Pak1 had little effect; depletion of both together had greater effect than depletion of Pak2 alone. It is concluded that Pak1/2 are activated during myoblast differentiation; that one way they are activated is via cell-cell adhesion; and that Pak2 promotes differentiation, with Pak1 playing a lesser role.

Notes

Canonical Wnt signaling in early and late mouse myogenesis

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1. The -58kb distal enhancer controls Myf5 expression in muscle progenitor cells of the limbs. It contains a potential binding site for Tcf/Lef1 transcription factors suggesting that Wnt signaling may play a regulatory role. We tested this hypothesis in a conditional β-catenin KO mouse generated with Myf5DE–Cre (distal enhancer). Homozygous mutants showed significantly reduced skeletal muscle mass and less motor activity than wild type mice. Muscle development in mutant embryos appeared unaffected until E14 when muscles began to decrease in size compared to wild type. M. latisssimus dorsi and M. subcutaneous trunci were affected most severely. At E18.5 the substantial reduction of Pax7+ cells was observed in mutants. Postnatal limb muscles of mutant mice contained less type I and IIA fibers that were mainly replaced by fast twitch IIB fibers. Taken these observations together we conclude that formation of secondary appendicular muscles, the number of Pax7+ presumptive satellite cells, and the normal distribution of oxidative and mixed oxidatve/glycolytic fibers in postnatal muscle are regulated by canonical Wnt signaling.
2. The distal (DE) and the early epaxial enhancer (EEE) activate specifically the Myf5 promoter but fail to interact with the Mrf4 promoter. To determine regions within the Mrf4/Myf5 locus that are required and sufficient to confer promoter specificity to these enhancers, we generated transgenic mouse lines containing dual reporter constructs. Our results suggest that neither enhancer is specifically selected by the Myf5 promoter but specificity is provided by a repressive mechanism associated with the Mrf4 promoter and sequences upstream of it. These elements seem to confer a competitive disadvantage to the Mrf4 promoter thus favoring enhancer interaction with Myf5.

Notes

Regulation of MRF expression in facial muscle precursors.

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During embryonic development precursor cells of different mesodermal origins follow distinctive routes to form skeletal muscles. Most facial muscles derive from posterior prechordal mesoderm, extraocular muscles from anterior prechordal mesoderm and a small number of mandibular muscles derive from splachnic mesoderm. Posterior prechordal mesodermal precursor cells first migrate into the branchial arches where they activate the myogenic cascade and develop into the muscles of mastication and of facial expression.
Five separate elements control the expression of Myf5 in craniofacial musculature but their individual contributions to the full pattern have not been elucidated. Transgenic analyses of several deletion constructs lacking one or more of these elements show that they contribute to a complex pattern of expression by activating and/or maintaining gene expression levels. Therefore, no single enhancer is responsible for the transcriptional regulation of Myf5 in particular aspects of the developing craniofacial musculature but an interplay of different activities constituting a fine network underlies the establishment of the pattern. Detailed analyses of one of these enhancers identifies Musculin (Msc, MyoR) as a crucial player in the maintenance of expression levels during early development. Importantly, we show that the regulation of MyoD in the branchial arches also follows this mechanism and that, once again, Msc is involved in establishing the correct expression levels. The existence of this network, in which enhancer function is regulated spatially and temporally, relies on the differential contribution of each element at various times and locations during development.

Notes

Regulation of skeletal muscle insulin signaling by the nuclear receptor, RORalpha.

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Retinoic acid receptor-related orphan receptor(ROR) alpha1 is a member of the Steroid-Nuclear hormone Receptor (NR) superfamily, i.e. DNA binding proteins that translate hormonal, nutritional and pathophysiological signals into gene regulation. We have previously demonstrated that ROR a1 deficient homozygous staggerer mice (sg/sg) are resistant to diet-induced obesity, and hepatic steatosis (Lau P et al. JBC, 283:18411-, 2008).
Skeletal muscle, a major mass peripheral tissue accounts for approximately 40% of the total body mass and significant levels of glucose disposal, fatty acid oxidation, and energy expenditure. Accordingly, we have utilised a skeletal muscle specific RORalpha dominant negative transgenic line, and the ROR a1 deficient/homozygous (and heterozygous) sg/sg mouse model to investigate lipid, glucose and energy homeostasis in skeletal muscle.

Our studies demonstrated that RORalpha1 expression controls blood glucose levels, glucose tolerance, insulin signaling and glucose uptake. Interestingly, increased insulin sensitivity in homo- and hetero-zygous staggerer mice was independent of (i) the adiposity (and weight), and (ii) of alterations in muscle fibre type. Profiling analysis demonstrated that ROR alpha signaling regulated: (i) NR signaling, (ii) lipid, glucose and energy homeostasis, (iii) metabolic disease, and (iv) PI3K/Akt and AMPK signaling. Whole body metabolic analysis suggested that respiratory exchange ratio (RER), activity, food intake (but not O2 consumption) were similar. Mechanims involved, regulation of Akt2 mRNA, protein and insulinmediated phosphorylation [Lau P et al., Diabetologia 2011(in press); Raichur S et al., NAR 38:4296-, 2010)]. In conclusion, we propose that Rora has a significant role in the regulation of skeletal muscle glucose uptake.

Notes

Basic regulatory controls of muscle gene expression and their applications to muscle cell and gene therapy.

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This talk will summarize recent experiments focused toward understanding regulatory controls of the M-creatine kinase gene (*MCK*), and their extrapolation to the control of many other skeletal muscle genes. These studies have utilized quantitative proteomics to identify novel candidate transcription factors: MAZ and KLF3 that play important roles in the activation of *MCK* during terminal differentiation *(Himeda, et al., Mol Cell Biol. 20: 6521; 30: 3430)*. Both factors exhibit their own transcriptional up-regulation coincident with skeletal muscle differentiation, and then associate with control elements in many different skeletal muscle genes. KLF3's mechanism of muscle gene activation is novel in that it contains an active transcriptional repression domain and has no known transcriptional activation domain. KLF3 associates with SRF, but not with MEF2s or MRFs, and the KLF3-SRF complex uses KLF3 DNA binding sites and SRF's transcriptional activation properties to enhance muscle gene expression.

Other studies have focused on characterizing a transcriptional enhancer located within *MCK* intron-*1 (Tai, et al., + Fischer-Aylor & Wold Skeletal Muscle, in press)*. These studies have identified a 95-bp enhancer containing a paired E-box/MEF2 regulatory motif whose activity in differentiating skeletal muscle is equivalent to that of the MCK gene's 5′ enhancer. ChIP and ChIP-Seq analysis indicate that the SIE, as well as the *MCK* 5′-enhancer, are occupied by MyoD, myogenin, and MEF2; whereas, many other E-boxes within or immediately adjacent to intron-1 are not occupied by MyoD or myogenin. Transgenic analysis of a 6.5-kb *MCK* genomic fragment containing the 5′-enhancer and proximal promoter plus the 3.2-kb intron-1, with and without its enhancer, indicate that the enhancer is critical for *MCK* expression in slow- and intermediate-twitch (Type-I and IIa) muscle fibers, but is not required for expression in fast-twitch (Type-IIb and IId) fibers.

Optimal gene and cell therapy for neuromuscular diseases will require the regulated expression of many different therapeutic proteins and regulatory RNAs. Depending on the disease and treatment strategy, these will need to be expressed in a variety of target cells: nascent myotubes, mature muscle fibers and cardiomyocytes, as well as in stem cells that have been directed into myogenic lineages. An added complexity is that fast and slow twitch muscle fibers, as well as different anatomical muscles, exhibit remarkably different expression levels of the same enhancer/promoter combinations.

The talk will describe how basic information from *MCK* and other muscle genes is used in designing regulatory cassettes. A critical technical component for optimal cassettes is their size compatibility with therapeutic cDNAs in conjunction with packaging in small viral vectors such as AAV. This is particularly relevant with cDNAs such as micro-dystrophin since its ~ 4kb size constrains the regulatory complexity that can be built into the cassettes. Strategies for the successful design of muscle-specific cassettes to achieve these ends, and comparative data from in vitro and in vivo studies will be presented. Several of the newest regulatory cassettes exhibit expression levels equivalent to that of powerful constitutively expressed cassettes such as CMV.

Notes

MicroRNAs in myogenesis: from physiology to pathology and back.

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During myogenesis the MyomiRs miR-1 and miR-206 are transcribed with a different timing, with miR-206 reaching maximum level at the onset of differentiation and miR-1 coming up later but remaining high in differentiated myotubes. Both microRNAs are not expressed in rhabdomyosarcoma (RMS). We have previously shown that rescue of miR-206, which shares the seed sequence with miR-1, can block the growth of RMS xenografts by inducing a major switch in the global gene expression profile toward that of mature muscle. Based on these results we proposed that miR-206 could be a useful tool for differentiation therapy of RMS. To follow up in this direction we decided to test the effect of rescuing expression of both miR-206 and miR-1 in RMS cell lines. Here we show that inducing expression of both MyomiRs has a dramatic effect in RMS cells in culture, bringing their myogenic conversion close to 90%. Furthermore, when rescuing miR-206 alone, upon withdrawal of the inducer the tumor resumed proliferation, indicating that a fraction of the RMS cells did not achieve terminal differentiation. Conversely, induction of both MyomiRs delayed tumor relapse upon removal of the inducer, allowing achievement of terminal differentiation in a higher number of tumor cells. Expression profiles of RMS cells converted by mir-206, miR-1, or by a combination of the two, indicate distinct transcriptional responses.

Notes

MicroRNAs confer robustness to developmental timing of myogenesis

Andrea Münsterberg, Katarzyna Goljanek-Whysall, Dylan Sweetman, Tamas Dalmay
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Commitment of progenitor cells to the myoblast fate in response to developmental cues is the first step in establishing the body musculature. Myogenic progenitors in the dermomyotome of developing somites express Pax3, a crucial transcription factor important for skeletal myogenesis. Following delamination from the dermomyotome it is essential to downregulate Pax3 to initiate the myogenic program including upregulation of myogenic regulators (MRFs). We investigated a role for muscle specific microRNAs, miR-1/miR-206 in regulating the progenitor to myoblast transition. We show that inhibition of miR-1/miR-206 using antagomirs leads to delayed myogenic differentiation in developing somites. This correlates with increased Pax3, which we show is directly targeted by miR-1 and miR-206. Pax3-specific target protector morpholinos phenocopy the antagomir-induced delay in muscle differentiation, suggesting that miR-1/miR-206 control somite myogenesis primarily through interactions with Pax3. Consistent with this idea, the antagomir induced loss of myogenin was rescued by Pax3 knock-down, using a splice-morpholino. Our studies reveal an important role for miR-1/miR-206 in providing precision to the timing of myogenesis in the embryo. We propose that post-transcriptional control of Pax3 by miR-1/miR-206 is required to stabilize myoblast commitment and subsequent differentiation.

Notes

Epigenetic control of miRNA expression through the dystrophin-nNOS signalling cascade

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microRNAs are recognized as important regulators of gene expression in the differentiation commitment of several cell types and have been shown to occupy very high hierarchical positions in the cascade of regulatory events controlling cell specification. Proper muscle tissue development and function was also described to depend on the controlled expression of specific families of miRNAs; in several cases it has been also shown that their ectopic expression can direct cells towards specific differentiation programmes. Moreover, altered levels of miRNAs were found in several muscular disorders such as myocardial infarction, Duchenne Muscular Dystrophy and other myopathies.
Duchenne Muscular Dystrophy is a severe genetic disorder caused by mutations in the dystrophin gene. The disruption of the Dystrophin-Associated Protein Complex at the muscle membrane, due to dystrophin deficiency, represents the primary event that leads to the disease pathogenesis: muscle fiber breakage and degeneration is then accompanied by a complex series of events including activation of satellite cells, inflammatory infiltration and intense fibrosis.
Even though a cure is not yet available, several different therapeutic strategies are nowadays entering human experimentation. In particular, exon skipping has been proven to be very powerful in restoring dystrophin expression and conferring benefit in animal models. Surprisingly, the beneficial effects observed on muscle function and morphology could be obtained also with low levels of protein rescue, suggesting that partial protein re-localization to the membrane could be only part of the story.
Taking advantage of a controlled rescue of dystrophin synthesis through exon skipping in mdx mice, we discovered that molecular circuitries, important for muscle differentiation and tissue integrity, such as oxidative stress and fibrosis, are directly controlled by dystrophin through NO signalling and epigenetic modulation of miRNA expression.
We also identified a miRNA (miR-31), highly expressed in Duchenne muscles, that represses dystrophin expression by targeting its 3'UTR. In human DMD myoblasts treated with exon skipping, we demonstrated that miR-31 inhibition increases dystrophin rescue. These results indicate that interfering with miR-31 activity can provide an ameliorating strategy for those DMD therapies aimed at efficiently recover dystrophin synthesis. Finally, we have shown that muscle miRNAs can represent suitable DMD serum biomarkers.

Notes

Epigenetic regulation of muscle regeneration by HDAC-regulated miRNA-SWI/SNF circuitry

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Dulbecco Teletohn Institute (DTI)

Dulbecco Telethon Institute (DTI), IRCCS Fondazione Santa Lucia, Rome, Italy and Sanford-Burnham Medical Research Institute, La Jolla, US

We have discovered that histone deacetylases (HDACs) are a crucial link between specific genetic mutations that cause muscular dystrophies and downstream determinants of disease progression. A large amount of experimental evidence demonstrate the key role of HDACs in the control of the transcriptional networks underlying the potential of dystrophic muscles to either activate compensatory regeneration or undergo fibro-adipogenic degeneration. We have identified a HDAC-regulated network that controls the expression of specific miRNAs, which target structural components (namely the Brg1/Brm-associated factors – BAFs) of the SWI/SNF chromatin remodeling complex to form sub-complexes containing mutually exclusive BAF60 variants that direct the myogenic or the fibro-adipogenic program in a novel population of muscle-derived pluripotent cells. This unanticipated regulatory axis is an important "epigenetic disease modifier", since it provides the restriction point for dystrophic muscle decision to either undergo compensatory regeneration or adipose infiltration and fibrosis - two deleterious events in the progression of muscular dystrophies.
These results illustrate a new link between HDAC, chromatin remodelers and the fate decision of muscle-derived pluripotent cells, and identify key targets for pharmacological interventions in the treatment of muscular dystrophies.

Notes

A chromatin-associated RNA regulates a Polycomb/Trithorax epigenetic switch at the basis of facioscapulohumeral muscular dystrophy (FSHD).

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More than 50% of the human genome is composed of repetitive sequences. Understanding what role these elements have in complex genomes is paramount.
FSHD is an autosomal dominant disease associated to contraction of a macrosatellite repeat, termed D4Z4, mapping to 4q35. By an unknown mechanism, D4Z4 deletion causes an epigenetic switch leading to de-repression of 4q35 genes in FSHD patients. Repression by D4Z4 displays a number of similarities with epigenetic gene silencing mediated by Polycomb group (PcG) proteins. Polycomb and Trithorax group (TrxG) proteins are essential for the proper development of multicellular organisms and work as counteractors in the epigenetic regulation of gene expression.
Here we found that core components of PRC1 (Bmi1 and Ring1B), PRC2 (Eed, Ezh2, Jarid2 and Suz12) and the associated repressive histone marks (H2AK119Ub and H3K27me3) are located at D4Z4 when 4q35 genes are repressed. Interestingly, 4q35 gene de-repression is associated to and requires recruitment of TrxG proteins to D4Z4.
In Drosophila, transcription of PcG-binding sites regulates their activity. Intriguingly, we found that D4Z4 generates a chromatin-bound, 4q35-associated RNA selectively in FSHD patients. Notably, this RNA regulates the chromatin structure at 4q35 and is required for 4q35 gene de-repression.
We propose that the RNA functions by recruiting TrxG to counteract PcG silencing at D4Z4 leading to 4q35 gene de-repression in FSHD.
Our results provide a critical nexus to reveal the epigenetic basis of FSHD etiology and to identify targets for developing possible therapeutic strategies. Moreover, this study is providing insights into the biological function of repetitive DNA elements in regulating gene expression and on how mutations of such elements can influence the progression of a human genetic disease.

Notes

Spatial re-organization of gene regulatory sequences temporally controls myogenic gene expression

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During skeletal muscle differentiation, the myogenic determinant MyoD promotes activation of genes expressed early in the differentiation process through recruitment of SWI/SNF ATP-dependent chromatin remodeling enzymes while simultaneously repressing genes to be expressed late in the differentiation process via recruitment of a histone deacetylase (HDAC). The molecular basis by which late expressed genes remain transcriptionally silent during the early stages of differentiation is not well understood. We show that the regulatory sequences, but not other regions of genes expressed at late times of myogenesis, are in close physical proximity in differentiating embryonic tissue as well as in differentiating culture cells, despite these genes being located on different chromosomes. 3C-Seq, a novel method to globally analyze inter- and intra-chromosomal interactions, reveals frequent genome-wide associations specifically between genes that are expressed in skeletal muscle. Formation of these interactions requires the Brg1 ATPase of SWI/SNF chromatin remodeling enzymes. Ectopic expression of myogenin and a specific Mef2 isoform induces skeletal muscle differentiation without activating endogenous MyoD expression. Under these conditions, the HDAC is not recruited to loci normally expressed at late times, the regulatory sequences of the loci are not in close proximity, and these genes are prematurely activated. The data indicate that MyoD-mediated spatial organization of late genes contributes to temporal regulation of myogenic transcription by restricting late gene expression during the early stages of myogenesis.

Notes

Molecular Regulation of Muscle Stem Cell Quiescence

Thomas Rando

We previous demonstrated that activation of the Notch pathway, primarily by Delta-like1 expression in the satellite cell niche activating Notch-1 on satellite cells, is essential for the activation of the satellite cell pool. Expression of the Notch inhibitor Numb leads to the down-regulation of Notch signaling necessary for myogenic differentiation. In order to explore in more detail the molecular regulation of satellite cell function by Notch signaling, we used a conditional gene deletion approach. We crossed a mouse strain in which the key transcriptional regulator in the Notch pathway, RBPj, was flanked by loxP sites with a strain bearing an inducible form of Cre recombinase (CreER) knocked into the Pax7 locus. Administration of Tamoxifen to adult offspring bearing both alleles results in the deletion of RBPj exclusively in satellite cells. Analysis of Notch target genes in purified, quiescent satellite cells from these mice revealed that they were almost all down-regulated compared to cells from control mice. Among the consequences of disruption of Notch signaling in quiescent satellite cells is their gradual depletion without any evidence of apoptosis. Rather, the cells appear to undergo spontaneous activation. Analysis of the kinetics of depletion suggests that active Notch signaling is essential for the maintenance of satellite cell quiescence and that the aberrant activation is associated with premature differentiation and the failure of self-renewal. In a parallel study, we conditionally deleted Dicer in satellite cells using the same Cre driver and a strain with a floxed Dicer allele. Similar to what was seen with RBP-J deletion, deletion of Dicer resulted in spontaneous satellite cell activation, but in this case the activated cells underwent apoptosis, resulting a depletion of satellite cells from the muscle. By miRNA microarray analysis, a group of quiescence-specific miRNAs was identified. Current studies are focusing on the role of individual miRNAs as well as downstream targets of those miRNAs that are essential regulators of satellite cell quiescence and activation.

Notes

MyoD gene suppression by Oct4 is required for reprogramming in myoblasts to produce induced pluripotent stem (iPS) cells

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Expression of the four transcription factors; Oct4, Sox2, cMyc and Klf4 has been shown to generate induced pluripotent stem (iPS) cells from many types of specialized differentiated somatic cells. It remains unclear, however, whether fully committed skeletal muscle progenitor cells have the potency to undergo reprogramming to develop iPS cells in line with previously reported cases. To test this, we have isolated genetically marked myoblasts derived from satellite cells of adult mouse muscles using the Cre-loxP system (Pax7-CreER:R26R and Myf5-Cre:R26R). Upon infection with retroviral vectors expressing the four factors, these myoblasts gave rise to myogenic lineage tracer lacZ-positive embryonic stem (ES) cell-like colonies. These cells expressed ES cell-specific genes and were competent to differentiate into all three germ layers and germ cells, indicating the successful generation of myoblast-derived iPS cells. Continuous expression of the MyoD gene, a master transcription factor for skeletal muscle specification, inhibited this reprogramming process in myoblasts. In contrast, reprogramming myoblasts isolated from mice lacking the MyoD gene led to an increase in reprogramming efficiency. Our data also indicated that Oct4 acts as a transcriptional suppressor of MyoD gene expression through its interaction with the upstream enhancer region. Taken together, these results indicate that suppression of MyoD gene expression by Oct4 is required for the initial reprogramming step in the development of iPS cells from myoblasts. This data suggests that the skeletal muscle system provides a well-defined differentiation model to further elaborate on the effects of iPS cell reprogramming in somatic cells.

Notes

The Pax7 expressing lineage

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Skeletal muscle tissue is prone to damage from acute physical trauma such as sport injuries as well as from daily wear and tear: this is managed by its tremendous capacity to repair itself. Cell transplantation and lineage tracing studies have demonstrated that Pax7 expressing adult satellite cells can repeatedly repair damaged muscle tissue after several bouts of acute injury: evidence that they are muscle stem cells (Sacco et al., Nature 456: 502-506, 2008; Lepper et al., Nature 460: 627-631, 2009).
Using temporally controlled inducible lineage tracing technique, we have constructed a developmental fate map of Pax7 expressing cells. Pax7 expressing dermomyotome cells at E9.5 have at least three fates, the dorsal dermis, the brown adipose tissues, and the muscle. Gradually, they become restricted to only muscle lineage at E12.5. We also determined that embryonically labeled Pax7 expressing cells do directly contribute to adult satellite cells capable of participating in muscle regeneration. Although our data indicate that there are emerging cells turning on Pax7 during embryogenesis, we are unable to find measurable contribution from de novo Pax7 expressing satellite cells after post-natal day 5 into the adult satellite cell pool.
However, it remains unclear whether there exist 'precursor' cells turning on Pax7 during the acute injury/regeneration process. This issue is further raised by the findings that stem cells from a variety of other origins are also reported to contribute to myofibers upon engraftment into muscles, questioning that Pax7 expressing satellite cells are not the only stem cell source for muscle regeneration. To resolve this issue, we have engineered genetic ablation of Pax7 expressing cells and determined whether there is any significant contribution to muscle regeneration from cells other than from this source. Unexpectedly, we find that elimination of Pax7 expressing cells completely blocks regenerative myogenesis either following injury to the tibialis anterior (TA) muscle or after transplantation of extensor digitorum longus (EDL) muscles into nude mice. As Pax7 is specifically expressed in satellite cells, we conclude that they are the sole source of myogenic stem cells for acute injury induced muscle regeneration in vivo. While technical manipulation in vitro can undoubtedly convert certain cells into muscle stem cells upon transplantation, our results should focus future research efforts on improving the usage of satellite cells in stem cell based therapy for muscular dystrophies.

Notes

Molecular Regulation of Muscle Stem Cell Function

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Satellite cells are a heterogeneous population composed of stem cells and committed progenitors. We undertook a gene expression analysis of satellite stem cells towards identifying signaling pathways that regulate their function. Our experiments identified the non-canonical Wnt-receptor Fzd7 as being specifically expressed in quiescent satellite stem cells. Wnt7a was examined as a candidate receptor for Fzd7 because of its expression and role during embryonic and adult myogenesis. We found that Wnt7a was markedly upregulated in newly formed myofibers during regenerative myogenesis, and that the Fzd7 receptor is necessary for Wnt7a binding at the surface of myogenic cells. Recombinant Wnt7a protein dramatically stimulated the symmetric expansion of satellite stem cells and that this expansion required Fzd7 and Vangl2, both components of the planar cell polarity (PCP) signaling pathway. Moreover, Wnt7a induced polarized localization of Vangl2 at opposite poles in pairs of dividing cells, in a manner consistent with Wnt7a activating PCP signaling. Over expression of Wnt7a during muscle regeneration resulted in an impressive enhancement of the regeneration process, generating more fibers of bigger caliber, independent of an effect on myoblast proliferation or differentiation. Importantly, Wnt7a over-expression resulted in a large expansion of the satellite stem cell population, and Wnt7a deficiency resulted in impaired maintenance of the satellite cell compartment. Therefore, Wnt7a signaling through the planar cell polarity pathway controls the homeostatic level of satellite stem cells and hence regulates the regenerative potential of muscle.

Notes

The regulation of muscle satellite cell fate choice

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Satellite cells are the resident stem cells of skeletal muscle. Since muscular dystrophies are characterised by muscle wasting, satellite cell function is likely compromised. A common disorder is Facioscapulohumeral muscular dystrophy, which is caused by a contraction in repeat units on chromosome 4q35. This contraction is thought to result in aberrant expression of DUX4, a homeodomain containing transcription factor. DUX4 inhibits myogenic differentiation and induces apoptosis, and was reported to increase levels of the transcription factor Pitx1 in C2 myogenic cells.
We have been examining the effects of DUX4 on satellite cells, where it also inhibits differentiation. In our hands, Pitx1 levels were unchanged, while Pitx2a and Pitx2b were up-regulated. This prompted us to examine further, the role of Pitx genes in satellite cell function. Pitx1 was undetectable, whilst Pitx2a, Pitx2b and Pitx2c were all low in proliferating satellite cells but increased during early differentiation. By contrast, proliferating cells expressed Pitx3, with levels then decreasing as cells differentiated.
Since DUX4 elevated Pitx2a and Pitx2b levels, we modelled this using retroviral-mediated constitutive expression. Pitx1, the Pitx2 isoforms and Pitx3, all had a similar effect: satellite cell proliferation was curtailed and the cells underwent precious differentiation. Conversely, siRNA-mediated gene knockdown resulted in an inhibition of differentiation.
Mircoarrays on satellite cells expressing DUX4 reveal multiple transcriptional changes, and it seems likely that DUX4 does not act primarily via Pitx, since this would promote, rather than inhibit, myogenic differentiation.

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Notes

Srf-dependent paracrine signals produced by myofibers control satellite cell-mediated skeletal muscle hypertrophy

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Adult skeletal muscles adapt their fiber size to their workload. We show that Serum Response Factor (Srf) is required for satellite cell-mediated hypertrophic muscle growth. Deletion of Srf from myofibers and not satellite cells blunted overload-induced hypertrophy and impaired satellite cell proliferation and recruitment to pre-existing fibers. We reveal a gene network in which Srf within myofibers modulates Il6 and Cox2/Il4 expressions and therefore exerts a paracrine control of satellite cell functions. In Srf-deleted muscles, in vivo over-expression of Il6 is sufficient to restore satellite proliferation but not satellite cell fusion and overall growth. In contrast Cox2/Il4 over-expressions rescue satellite cell recruitment and muscle growth without affecting satellite proliferation, identifying altered fusion as the limiting cellular event. These findings unravel a novel role for Srf in the translation of mechanical cues applied to myofibers into paracrine signals, which in turn will modulate satellite cell functions and support muscle growth.

Notes

Identification of epigenetic modifiers involved in muscle stem cell differentiation

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Skeletal muscle stem cells or satellite cells are situated on myofibers in dormant state but can be activated by physical exercise and injuries of the muscle thereby responding to physiological and pathological needs and maintaining muscle homeostasis. Satellite cell differentiation is accompanied with massive chromatin reorganization indicating that epigenetic mechanisms control muscle stem cell homeostasis. To identify chromatin modifiers required for muscle stem cell activation and differentiation we performed proteomic analysis on a FACS purified population of satellite cells from transgenic Pax7-Cre/ZEG reporter mice and obtained a proteome of satellite cells with a depth 4500 proteins. Among them we found histone arginine methyltransferase Prmt5 to be exclusively expressed in satellite cells but not in differentiated myonuclei, which is confirmed by immunoflurescence in vivo using isolated myotubes. Importantly symmetric histone H4 methylation (H4R3me2), the histone modification catalyzed by Prmt5, is enriched in resting and self-renewing satellite cells and down-regulation of Prmt5 leads to muscle stem cell differentiation. Our results indicate that Prmt5 mediated H4R3me2 maintains muscle stem cells in the undifferentiated state.

Notes

Satellite cell properties and function

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After embryonic development is complete, a pool of juvenile and adult satellite cells is allocated for further skeletal muscle growth and regeneration postnatally. These are the stem cell population of the tissue, yet numerous studies have indicated that this population is herteogeneous, comprised of "stem-like" and committed cells, and other cell types including PICs and mesoangioblasts have reported to have muscle regenerative capacity. We examined the properties of satellite cells in the context of exit from quiescence and division rates in vivo and in vitro. We show that satellite cells enter the cycle, initially with a long lag period, followed by a relatively fast cell cycle time, and in a synchronous manner. However, heterogeneity is observed with respect to the lag period for exit from quiescence. We show also that the major effector of Notch signaling, Rbpj, is a key player is regulating satellite cell quiescence. Specific elimination of Rbpj during homeostasis or after muscle injury results in the depletion of the satellite cell pool and failed muscle regeneration. This phenotypic outcome is similar to a satellite cell ablation model using diphtheria toxin (in collaboration with Anne Galy, Genethon), where elimination of satellite cells results in failed regeneration. The failure of other regenerative cell types in skeletal muscle to effect muscle repair in the absence of satellite cells leads us to propose that satellite cells are necessary for orchestrating their muscle regenerative properties, possibly via the secretion of effector molecules

Notes

Satellite cell quiescence during growth and regeneration

Andrew Brack , Rana Abou-Khalil , Joe Chakkalakal
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Satellite cells (SCs) are the principal source of progenitors for muscle growth and regeneration. Satellite cells undergo asymmetric fate decisions to maintain muscle tissue and the SC pool, respectively. Adult stem cell compartments contain a relatively slow diving subset that retains high self-renewal capacity. It remains unknown whether the self-renewing adult satellite cell pool contains a slow dividing subset. Therefore we examined if SCs possess a slow dividing subset. To this end, we used a doxycyline (dox)-inducible H2B-GFP reporter mouse strain to study and quantify SC turnover during homeostasis and regeneration in vivo. After dox feeding, nearly all quiescent adult SCs express uniform levels of GFP. Upon dox removal, GFP levels decline 50% with each cellular division. This system provides a quantifiable readout of SC division history in vivo. Significantly, this can be used to initially label quiescent SCs and is subsequently partitioned equally in SC daughters, overcoming caveats of alternative methods such as BrdU labeling. Our studies indicate that the SC pool does not turnover as a uniform population in vivo. Rather, the self-renewing SC pool is composed of a fast and slow dividing population. Moreover, we demonstrate that proliferative history biases cell fate decisions involving self-renewal and differentiation. Our work demonstrates that the self-renewing SC pool is functionally heterogeneous based on its proliferative history.

Notes

Muscle Stem Cells Secrete Tenascin W, a Key Component of Their Niche

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We have generated a zebrafish GFP reporter line for pax7, a known master regulator of muscle stem cell (MSC) physiology. This allowed us to study the dynamics and cellular architecture of the myogenic lineage by non-invasive 4D in vivo imaging during primary and secondary myogenesis. The analysis of overlapping imaging sequences allowed us to obtain the full complement of MPs and showed that they derive from a population of MSCs that originate in the teleost dermomyotome. Clonal analysis combined with anatomical, morphological and functional criteria led to the separation of quiescent and activated MSCs from Pax7+ early myoblasts and concomitantly identified the vertical myoseptum (VM) as the niche of zebrafish MSCs. Furthermore it allowed us to obtain estimates for the cell cycle time of these subpopulations during physiological muscle development and muscle regeneration.
By transcriptional profiling we have found that MSCs play a pivotal role in the ontogeny of the VM and hence in the construction of their own niche by secreting a number of non-collagenous ECM components including tenascin w (tnw). Upon morpholino-mediated knockdown of tnw we observe illegitimate activation of the quiescent MSC population, a dramatic decline of the total MSC number as well as structural defects of the VM and muscle fibre disorganization.
Currently, we are screening a small set of Wnt, BMP, FGF and TGFb signalling inhibitors for their effect on myogenic lineage dynamics.

Notes

Linking transcription factor occupancy and chromatin state to gene expression during embryonic development

Eileen Furlong

One of the central challenges in biology is to understand how the genome is utilized to give rise to diverse cell types. Embryonic development occurs through the progressive restriction of cell fates, from pluripotent fields of cells to complex organs and tissues. This process requires a directed progression through interlinked regulatory states, each defined by the total set of active transcription factors. At each stage of development, the combined inputs of signalling and transcriptional networks regulate the expression of specific sets of genes that drive the transition to the next, often more specialized, state. Understanding how the underlying cis-regulatory networks produce spatial and temporal gene expression is therefore an essential step towards deciphering metazoan development and ultimately evolutionary change.
While genetic studies have identified a growing number of essential transcription factors (TFs) required for cell fate specification, little is known about the mechanisms by which these regulators function. Conversely, recent global approaches assaying TF binding enable the location and even combinatorial occupancy of CRMs to be experimentally measured as specific stages of development, at a genome-wide scale. A current major challenge is to interpret these TF binding data in terms of their resulting spatio-temporal cis-regulatory activity. Our work attempts to bridge this gap, by integrating genetic, genomic and computational approaches to understand how transcriptional networks drive cell fate selection, using mesoderm specification in Drosophila as a well-defined model system. I will present our current status on an on-going effort to build a predictive cis-regulatory network defining multiple stages of development.

Notes

Comparison of the Myogenic and Neurogenic Regulatory Programs

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The molecular mechanisms involved in the establishment of unique committed lineages by bHLH proteins remain incompletely elucidated. To gain insight into how differentiation program specificity is mediated by different bHLH proteins, we compared the genome-wide binding profiles of MYOD and NEUROD2. Comparison of muscle differentiation mediated by MYOD in fibroblasts and neuronal differentiation mediated by NEUROD2 in P19 cells demonstrates an expected set of unique binding sites and gene targets, as well as an intriguing set of commonly bound sites and overlapping gene targets that is suggestive of a core differentiation program used during lineage commitment. We also show that while binding is limited to E-boxes found within an accessible chromatin context, additional factors serve to modulate binding within these areas of open chromatin. These include a strong E-box sequence preference for MYOD and NEUROD2, as well as the presence of other factor-binding motifs. Together, our findings indicate how MYOD and NEUROD2 selectively bind throughout the genome in order to activate both common and specific genes required for execution of distinct differentiation programs.

Notes

A novel strategy for autologous cell therapy for Duchenne Muscular Dystrophy

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Mesoangioblasts are recently characterized progenitor cells, associated with the vasculature and able to differentiate into different types of solid mesoderm, including skeletal muscle (1).
When intra-arterially delivered to dystrophic muscle of dystrophic mice and dogs, mesoangioblasts resulted in a significant functional amelioration (2,3).
Human adult mesoangioblasts were characterized (4) and are currently used for a phase I/II study of donor cell therapy in DMD patients. This requires immune suppression and availability of an HLA-matched donor. To overcome these limitations we are using a human artificial chromosome containing the whole dystrophin gene (HAC-Dys) that we transferred in mouse dystrophic mesoangioblasts. Corrected dystrophic mesongioblasts were transplanted in mdx/SCID mice where they readily engrafted, expressed human dystrophin and ameliorated the phenotype for many months after the injection. In the case, of human mesoangioblasts, cells were reversibly immortalized by transduction with floxed lentivectors expressing human telomerase reverse transcriptase (hTERT), and Bm-1, together with the suicide gen HSTK. Immortal cells maintain telomerase activity and long telomere while remaining untransformed, unable to geneate tumors and able to terminally differentiate in vitro. The HAC-Dis has been transferred in these cells and two clones have been selected which contain a copy of the HAC. They will be transplanted into mdx/SCID mice before and after Cre-mediate excision of integrating vectors. Future development foresees a second generation HAC-Dys containing the dystrophin gene, floxed immortalizing sequences (hTERT-Bmi-1-HSTK), an inducible MyoD (MyoD-ER) and four full-length human dystrophin cDNAs to compensate gene dosage insufficiency deriving from fusion of relatively few genetically corrected cells in a fiber containing a large majority of genetically defective nuclei. If successful, this project will set the stage for a definitive cell therapy of DMD.

1. Minasi et al. Development 129, 2773, 2002
2. Sampaolesi et al. Science 301, 487, 2003
3. Sampaolesi et al. Nature 444, 574, 2006
4. Dellavalle et al. Nature Cell Biol. 9, 255, 2007

This work is supported by grants from the European Community, the European Research Council, Duchenne Parent Project, Telethon and the Italian Ministries of Research and Health.

Notes

Therapy ideas to therapy development- streamlining the process of getting treatments for neuromuscular diseases

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Since the discovery of the gene responsible for Duchenne muscular dystrophy the underlying basis of many inherited neuromuscular diseases has been elucidated. As well as improving diagnosis, and giving the possibility of directed management, understanding the molecular pathology of these conditions has also led to the prospect for directed therapy. With therapeutic options for DMD leading the way, several trials have already been performed. These trials have addressed various potential therapeutic modalities including gene therapy and RNA modulation as well as small molecule development based on an enhanced understanding of the downstream pathology of the disease. With proof of principle established in some of these areas, bigger pharma are beginning to be engaged more in this space, which is an exciting development.
In a rare disease area, there are specific challenges that need to be addressed in facilitating the development of trials. Patients, often with particular subsets of mutations, need to be identified as do experienced trial sites with information about how to monitor disease progression. Networks such as TREAT-NMD have worked to establish the infrastructure to address these challenges and ensure that therapy development can reach this patient group, with the pathway also established for other rare inherited neuromuscular disorders for which a similar set of tools and resources can be applied.

Notes

Antisense-based exon skipping as a therapy for Duchenne Muscular Dystrophy, status and prospects

Gert Jan B. van Ommen
Leiden University Medical Center

Antisense-based exon skipping as a therapy for Duchenne Muscular Dystrophy, status and prospects
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Twenty years after the first wave of Mendelian disease gene discoveries, their translation into therapeutic modalities begins to take hold, greatly aided by the increase in biological insights and advancement of technologies of the genomics era. A good example is the application of antisense-mediated exon skipping. This is presently the most promising therapeutic approach for Duchenne muscular dystrophy (DMD): a successful phase I/II systemic clinical trial has been published and a multicenter phase III placebo-controlled clinical trial is ongoing. Our preclinical further research now focuses on the next steps in developing and improving therapy: the development of more refined readouts for therapeutic success using transcriptomics and proteomics technology, and supporting treatments to increase myogenesis. In parallel, the success of the exon skipping in DMD has led us to explore applications to other Mendelian diseases, like Huntington disease and CADASIL, but also to a more common disease, rheumatoid arthritis. Thus, the current high-troughput tools and technology increasingly assist a beneficial convergence of the fields of common, multifactorial, and rare, Mendelian disease, classically seen as largely separate. This synergism has much to contribute to better defining biology, predicting health outcomes and guiding therapy development.

Notes

Regulation of muscle cell fusion by actin nucleation

Benny Shilo [1], Yael Gruenbaum-Cohen [1], Boaz Gildor [1], R'ada Massarwa [1], Priyankana Mukherjee [2], K Vijay Raghavan [2], Eyal Schejter [1]

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Actin nucleation-promoting factors (NPFs) of the WASp and SCAR/WAVE protein families, trigger formation of branched actin arrays by activating the Arp2/3 complex. Our work focuses on elucidating the role of actin nucleation in muscle cell fusion, in a variety of biological settings. The Drosophila WASp and SCAR/WAVE homologs are both essential for fusion of fusion-competent myoblasts to myoblast founder cells and myotubes during fly embryogenesis. Our studies suggest that the two NPFs act sequentially, and mediate distinct actin-based functions during embryonic myoblast fusion. Furthermore, we have demonstrated a requirement for both NPFs during the second round of Drosophila myogenesis, which leads to formation of adult fly muscles. To study the role of the same machinery in muscle fusion in mice, the N-WASp gene was knocked out specifically in myoblasts prior to fusion. Complete elimination of fusion in all muscle types was observed. Primary muscle cell culture studies show that cell motility is retained in N-WASp mutant myoblasts. The activity of N-WASp is required in both cells of fusing myoblast pairs. Taken together, these studies show a universal role for branched actin nucleation in muscle cell fusion. Future work will examine the mechanistic basis for this activity.

Notes

DSwiprosin-1/EFhd2 accumulates in FuRMAS at the prefusion complex stage during Drosophila myoblast fusion

Renate Renkawitz-Pohl
phillips-universität Marburg

We analyse myoblast fusion during Drosophila embryogenesis as a model system, where founder cells (FCs) for each individual muscle fuse with a distinct number of fusion competent myoblasts (FCMs) to build an individual muscle. Transient cell adhesion between FC/growing muscles and FCMs leads to the formation of Fusion-Restricted Myogenic-Adhesive Structures (FuRMAS). FuRMAS share numerous features with the immunological synapse, podosomes and invadopodia such as transient adhesive rings, F-actin accumulation and branching (Kesper et al., 2007, Dev Dyn., 236:404-15; Önel and Renkawitz-Pohl, 2009, Dev Dyn., 238:1513-25; Önel et al., 2011, in: Cell Fusions ed. L.-I.+L. Larsson, Springer pp 139 - 170). We identified DSwioprosin-1 (DSwip-1), the Drosophila homologue of human and mouse calcium-binding proteins called Swiprosins. Within FuRMASs, DSwip-1 accumulates transiently in foci within fusion-competent myoblasts (FCMs). Both the EF-hand and the coiled-coil domain of DSwip-1 are required to localize the protein to the characteristic foci. Previously, we suggested that the vesicles of the prefusion complex (Doberstein et al., 1997, J Cell Biol., 136:1249-61) are localized within the centre of the FuRMAS. Analysing given fusion mutants, we found DSwip-1 foci to increase in number in sing22 mutants, which arrest fusion after the prefusion complex formation. In contrast, DSwip-1 foci are not significantly enriched in blow2 and ketteJ4-48 mutants, which stop myogenesis beyond the prefusion-complex stage but before plasma-membrane merging. Therefore, we hypothesize that the calcium-binding protein DSwip-1 participates in the devolvement of the prefusion complex during the progression of myoblast fusion (Hornbruch et al., in revision).

Notes

MAP and Kinesin dependent nuclear positioning is required for skeletal muscle function

Mary Baylies [1], Thomas Metzger [2], Vincent Gache [3], Mu Xu [1], Bruno Cadot [3], Eric Folker [1], Brian Richardson [1], Edgar Gomes [4]
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[4] Université Pierre et Marie Curie Paris and Groupe Hospitalier Pitié-Salpêtrière, Institut de Myologie

The basic unit of skeletal muscle in all metazoans is the multinucleate myofiber, along which individual nuclei are regularly positioned. The molecular machinery responsible for myonuclear positioning along the fiber is not known. Improperly positioned nuclei are a hallmark of numerous muscles diseases, including centronuclear myopathies, but it is unclear whether correct nuclear positioning is necessary for muscle function. We have developed systems to study nuclear positioning during myofiber development in Drosophila and in cultured mammalian myotubes. We identified the microtubule-associated protein Ensconsin(Ens)/MAP7 and Kinesin Heavy Chain (Khc)/Kif5b as essential, evolutionary conserved regulators of myonuclear positioning. We have mapped the domains of interaction between these proteins, identifying which regions are critically involved for nuclear positioning. Particularly, expression of the Kif5b motor domain fused to the MAP7 microtubule binding domain rescues nuclear positioning defects in MAP7 depleted cells, suggesting that MAP7 links Kif5b to the microtubule cytoskeleton to promote nuclear positioning. Drosophila ens mutant larvae display decreased locomotion and incorrect nuclear positioning, and these phenotypes are rescued by muscle specific expression of Ens. We conclude that improper nuclear positioning contributes to muscle dysfunction in a cell autonomous fashion. By linking intracellular organization with behavior, our systems provide a novel platform for investigating the fundamental mechanisms underlying nuclear positioning in muscle development/repair and the pathogenesis of muscle disease.

Notes

Comparative gene expression profiling identifies the tetraspanin CD53 as a p38α-dependent protein that modulates cell fusion

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Formation of multinucleated myotubes is mediated by a complex gene expression program that critically requires both the muscle-specific transcription factor Myogenin (Myog) and p38α MAPK signaling. Using multiple cell types (primary mouse myoblasts, C2C12 myoblasts, 10T1/2 fibroblasts), we determined that expression of Myog is modulated by p38α signaling during myogenesis. Based on these findings, we hypothesized that the critical role for p38α in myogenesis could be mediated through the regulated expression of myogenin. To address this possibility, we generated a C2C12 cell line that stably maintained an exogenous Myog cDNA under the control of a Tetracyclin inducible promoter. Combining this system with the use of a pharmacological inhibitor of p38α/β activity (SB203580), allowed us to establish Myog expression in the absence of p38α signaling. Using comparative analysis to interrogate the interdependency of p38α and Myog during myogenesis, we found that Myog expression (in the absence of p38α/β signaling) is sufficient to establish myoblast alignment and expression of multiple late markers of muscle differentiation. However, the absence of multinucleated myotubes in these conditions revealed a critical role for p38α MAPK in myoblast fusion. Analysis of p38α MAPK up-regulated genes led to the identification of the tetraspanin CD53 as a candidate fusogen. Importantly, knockdown of CD53 in myoblasts confirmed a role for this membrane protein in the formation of multinucleated myotubes. Thus, our comparative genomic approach has identified CD53 as a p38α MAPK-dependent gene that mediates cell fusion during myogenesis.

Notes

Skeletal myosin light chain kinase regulates skeletal myogenesis by phosphorylation of MEF2C

Ashraf Al-Madhoun , Virja Mehta , Grace Li , Daniel Figeys , Nadine Wiper-Bergeron , Ilona Skerjanc
University of Ottawa

The MEF2 factors regulate transcription during cardiac and skeletal myogenesis. MEF2 factors establish skeletal muscle commitment by amplifying and synergizing with MyoD, although lineage-specific regulation is unknown. Here, we show that phosphorylation of MEF2C on T80 by skeletal myosin light chain kinase (skMLCK) enhances skeletal and not cardiac myogenesis. A phosphorylation-deficient MEF2C mutant (MEFT80A) was able to enhance cardiac, but not skeletal myogenesis in P19 stem cells. Further, MEFT80A was deficient in recruitment of p300 to skeletal but not cardiac muscle promoters. In gain-of-function studies, skMLCK upregulated MRF expression leading to enhanced skeletal myogenesis in P19 cells and more efficient myogenic conversion. In loss-of-function studies, MLCK was essential for efficient MRF expression and subsequent myogenesis in P19 and embryonic stem cells as well as proper activation of quiescent satellite cells. Thus, skMLCK regulates MRF expression by controlling the MEF2C-dependent recruitment of histone acetyltransferases (HATs) to skeletal muscle promoters. This work identifies the first kinase that regulates MyoD and Myf-5 expression in ES and satellite cells.

Notes

Regulation of limb muscle morphogenesis

Peleg Hasson , Malcolm Logan
NIMR

For limbs to function properly each of the tissues that comprise the musculoskeletal unit-the bones, muscles and tendons-must be the correct size and shape and make the appropriate interconnections with one another. Although there is great complexity in the final pattern of connections of mature limb muscles with their associated tendons and bones, the limb musculature has a separate embryological origin from the other elements of the appendicular skeleton. Limb muscles are derived from precursors that migrate from their original axial location in the hypaxial dermomyotome into the forelimb and hindlimb buds that emerge from the lateral plate mesoderm. Although these precursors are apparently committed to their ultimate muscle fate the exact type of muscle that they form is determined by the environment into which they migrate. Muscle precursors that migrate into the forelimb bud will form forelimb muscles while muscle precursors that migrate into the hindlimb bud will form hindlimb muscles. Loose connective tissue, commonly referred to as muscle connective tissue, that surrounds the forming soft tissues is the apparent source of patterning cues. We have shown that the integrity of the connective tissue is critical for correct patterning of the soft tissues and that this is independent of patterning of the skeletal elements.

Notes

Dynamics of skeletal muscle cells during newt limb regeneration

Andras Simon [1], *Jamie Morrison, Sara Lööf, Heng Wang, Paula Borg*
[1] *Karolinska Institute*

Aquatic salamanders such as newts are exceptional among adult vertebrates as they can rebuild complex structures, such as entire limbs. I will address mechanisms by which skeletal muscle cells contribute to the new limb after amputation, focusing on three fundamental questions. First, I will discuss the relationship between stem cell activation and cellular dedifferentiation as two complementary ways of producing new limb cells. I will present data showing that limb regeneration is driven both by cellular dedifferentiation and by stem cell activation. Second, I will discuss whether skeletal muscle derived cells remain lineage restricted during limb regeneration. Here I will show results indicating that skeletal muscle derived cells have a potential to contribute to cartilage tissue. Third, I will describe a model by which an injury induced cell death response is used to reprogram terminally differentiated skeletal muscle cells. According to this model cellular survival is not always manifested by the return to the original phenotype but also by the production of a new cell type. This strategy may be a component of newts' ability to divert an injury evoked cell death response into mechanisms that fuel regeneration.

Notes

Reformation of Muscle and Skeleton during Axolotl Limb Regeneration

Elly Tanaka
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After limb amputation in Axolotls, a faithful version of the missing portion regenerates including skeletal elements and muscle. We have been tracking the process of muscle versus connective tissue/skeletal regeneration and observe a number of cellular and molecular differences in the regeneration of these two tissue compartments. Overall, limbs will regenerate only those limb segments more distal to the amputation plane, a phenomenon called "the rule of distal transformation". On the tissue level we find that cells of the skeleton and connective tissue display this restriction to form more distal limb structures. Correspondingly, these cell types appropriately express transcription factors associated with limb segment identity such as Meis and Hox homeodomain proteins in a spatially appropriate manner. In contrast, muscle does not obey the rule of distal transformation, so that lower arm derived muscle cells can contribute to upper arm muscle tissues. Interestingly, during early blastema formation, muscle derived blastema cells express these transcription factors, but in an inappropriate manner. We are currently testing whether this profile of transcription factor expression contributes to the ability of muscle to break the rule of distal transformation. I will also discuss our experiments tracking the fate of mature muscle fibers during limb regeneration.

Notes

p53 plays a pivotal role in preventing malignant transformation of induced pluripotent stem cells.

Rachel Sarig, Noa Rivlin, Ran Brosh, Chamutal Bornstein, Naomi Goldfinger, Ori Brenner, Varda Rotter

Somatic cells can be reprogrammed to induced pluripotent stem cells (iPSC), by ectopic expression of defined factors. iPSC are similar to embryonic stem cells (ESC) in morphology, proliferation, transcriptional profiles and pluripotency. Therefore, it is possible to envision numerous therapeutic applications for iPSC. However, the benefits of iPSC may be hampered by safety concerns such as their tumorigenecity. Reprogramming resembles closely the process of transformation. In both processes, somatic cells with limited proliferation capacity acquire indefinite self-renewal ability after extensive modifications, including profound changes to their epigenetic status. Reprogramming of somatic cells, which already carry defects in their tumor-suppressing machinery, may tilt their destiny towards malignant transformation. Indeed, in addition to the role played by the tumor-suppressor p53 in regulating the efficiency of reprogramming, we demonstrate a novel p53-dependent qualitative mechanism that controls the integrity of iPSC by keeping the balance between differentiation and malignant transformation*. We found that reprogramming of p53 deficient cells give rise to iPSC, which despite their typical ESC characteristics in vitro, form malignant tumors in vivo, suggesting a primordial role for p53 in maintaining genomic stability to ensure the fidelity of stem cells. Our data highlight the notion that iPSC with apparently normal features in vitro and with the capacity to differentiate into the three germ layers in vivo, could form malignant tumors, suggesting that in genetic instable cells, reprogramming may result in the formation of cancer rather than normal stem cells.

Notes

ABSTRACTS - POSTER PRESENTATIONS

(sorted by name of presenter)

Analysis of human satellite cells in single skeletal muscle fiber fragments

Stephanie Adams [1], *Sina Gloy* [1], *Katrin Wenzel* [1], *Josef Zacher* [2], *Andreas Spuler* [3], *Simone Spuler* [1]
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While our increasing knowledge on murine satellite cells has shed light on the mechanism of satellite cell function, the understanding of the human counterpart has remained very poor. Therefore, we aim to characterize the human satellite cells. To analyze their regenerative potencies, we established the mechanical isolation of human single fiber fragments. Cultured fibers were harvested in two day intervals up to 28 days of culture and analyzed for satellite cell and activation markers (Pax7, Myf5, MyoD, desmin). Pax7/Myf5 double positive cells defining early activated satellite cells were detectable after 4 days. Then, these cells proliferated and differentiated inside the fiber as they become desmin positive. Only upon destruction of the basal lamina outgrowth was observed. Evading and extensively proliferating cells generated almost pure myoblast populations of more than 1000 cells per fiber fragment (≥1cm) within 14 days, independent of the age of the donor (27-88 years of age; probands: n=25; fiber cultures: n=300). We also identified single cells that had regained Pax7 expression indicating asymmetric division and ability to repopulate the satellite cell pool even in older age. To summarize, we first describe the age-dependent human satellite cell activation kinetic in their intact homing niche. Furthermore, we demonstrate an enormous regenerative potency of adult human satellite cells, independent of the age of donor.

SoxF Genes as Important Transcriptional Regulators of Satellite Cell Self-Renewal

Sonia Alonso-Martin [1], *Anne Rochat* [1], *Frederic Aurade* [1], *Pete Zammit* [2], *Frederic Relaix* [1]
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Around birth, fetal muscle progenitor cells adopt a satellite cell position, becoming embedded within the basal lamina in close contact to the muscle fibers. Importantly, in addition to this morphological change, the emerging satellite cells enter quiescence, a molecular state poorly characterized in vivo. During post-natal growth or in response to injury or disruption of the basal lamina, a subset of the satellite cells become activated, proliferate and either fuse to form multinucleated myotubes or re-establish a residual pool of quiescent satellite cells that have the capability of supporting additional rounds of growth/regeneration.

We were interested in identifying new molecular pathways involved in the progression from a proliferating population to a quiescent post-natal progenitor cell population. Pax3 is a paired-homeobox transcription factor expressed in muscle progenitor cells throughout development, including post-natal satellite cells. We have previously generated a Pax3GFP/+ allele (Nature, 435, 948-953), which provides direct and efficient access to the Pax3-expressing muscle progenitor cells through FACS-sorting.

After performing expression profiling of Pax3GFP/+ cells during development and post-natally, we have identified new transcription factors, the SoxF genes, which are induced at the onset of satellite cells emergence. SoxF genes (Sox7, Sox17, Sox18) are members of the SOX family of transcription factors with a trans-activation domain C-terminal to the HMG box. They are involved in the regulation of embryonic development and in the determination of the cell fate. Results as well as functional analysis of these genes in the satellite cells will be presented.

Lateral inhibition mediated by Notch signaling underlies the segregation between striated and smooth muscle cells issued from the dermomyotome

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We aim at understanding the mechanisms that govern the segregation of lineages issued from the embryonic dermomyotome (DM). The DM forms from the dorsal aspect of the somite and remains epithelial after the dissociation of sclerotomal progenitors. The central domain of the DM gives rise to striated muscles and dermis of the back. The lateral aspect of the DM contributes to striated and smooth muscles and to endothelium. Lineage analysis of the latter region has shown that while segregation of endothelial precursors is an early event, the lateral DM still harbors single progenitors for both muscle fates. Notch signaling was shown to promote smooth muscle fate at the expense of striated muscle fate (Ben-Yair and Kalcheim, 2008). Inhibiting Notch signaling by overexpressing Numb favors striated muscle fate at the expense of smooth muscle fate. We here show that Dll1, a Notch ligand, cell-autonomously promotes striated muscle fate while inducing vascular fate in neighboring cells by activating Notch signaling in those cells. Dll1 activates a Notch reporter in adjacent cells 8 hours post-treatment. 16-20 hours post-treatment Notch reporter-positive cells are found primarily in the sclerotome, incorporating into the lining of blood vessels after 30 hours. We thus conclude that Notch signaling, via lateral inhibition, is involved in the segregation of either muscle fates.

Cf2 regulates Mef2 levels and participate in the regulation of the myoblast fusion process

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[3]

The Drosophila transcription factor Chorion factor 2 (Cf2), initially identified by its role in establishing the dorso-ventral pattern during oogenesis, is expressed in embryonic and adult skeletal muscles throughout development. However not much is known about its function in muscle differentiation. In this work we have analyzed the effects that perturbations in Cf2 expression levels produce in muscle formation during Drosophila development. This study has been carried out using gain or loss of function approaches to analyze the phenotypic changes that occur in embryo muscles after varying Cf2 expression levels.
In order to determine Cf2 specific role in myogenesis we have carried out functional studies. We demonstrate that Cf2 modulates Mef2 expression levels at the stages when both factors are co-expressed throughout embryonic development. Cf2 is able to modify both, Mef2 mRNA and protein levels, indicating the existence of a feedback mechanism between these two factors during muscle differentiation. Our results indicate that Cf2 expression levels must be tightly regulated since strong over-expression results in embryonic lethality. Moderate alterations of its expression levels, up or down regulation, induce significant changes in the number of nuclei per muscle fibre. Therefore, it disturbs the final size of the fibre as so does with its organization, morphology and, sometimes, total number of somatic fibres present in each hemisegment. Therefore, changes in Cf2 expression levels alter the embryonic muscle pattern. The mechanism by which this process occurs is currently unknown but our data suggest that Cf2 may participate in the regulation of the fusion process.

Endothelial derived progenitors in muscle development and regeneration

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Despite the increasingly recognized medical value of mesoangioblasts and other vessel-associated multipotent mesodermal progenitors, indirect extraction methods precluded the understanding of their native identity and developmental role. We tried to address this question by using a genetic lineage tracing approach.
At embryonic, foetal and perinatal stages, we detected skeletal muscle, smooth muscle and dermis cells originating from embryonic VE-Cadherin+ progenitors, and not from somites, as part of their in vivo normal developmental fate.
Consistently, we have observed a small number of endothelial-derived muscle fibers in the adult skeletal muscle. We have not detected any contribution to the satellite cell pool in basal conditions. However, a significant number of muscle interstitial CD31- cells appear to be originated by embryonic endothelial progenitors. Among them, subsets of pericytes and PICs (PW1+ interstitial adult myogenic progenitors) are labelled.
Following muscle damage, cells derived from embryonic endothelial progenitors participate to myogenic regenerative response, generating (or fusing to) myofibers. We have isolated endothelial derived cells by FACS from embryo and from

healthy and injured muscle. Endothelial derived cells are myogenic in vitro and are able to differentiate in several mesodermal tissues, including SMA (smooth muscle actin) positive cells, AP (alkaline phosphatase) positive osteoblast-like cells, adipose tissue and endothelial networks. Intra-muscular injection of sorted embryonic endothelial derived progenitors results in colonization and reconstitution of skeletal muscle tissue in both wild type and dystrophic mice.
These data suggest the existence of a previously unrecognized endothelial-derived progenitor cell subpopulation, which could represent the in vivo counterpart of embryonic mesoangioblasts.

Comprehensive analyses of noncoding RNAs in Polycomb-mediated myogenic differentiation.

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Epigenetic mechanisms play a major part in myogenesis. Polycomb group proteins (PcG) are epigenetic regulators able to maintain the repressive state of specific set of genes involved in cell fate determination by controlling chromatin structure. It is now established that they play a key role in the maintenance of the skeletal muscle progenitors' identity and that their dynamic regulation is crucial for terminal muscle differentiation.
Over the past few years, studies have also unravelled the ability of non-coding RNAs to directly interact with PcG proteins and to be necessary for gene silencing. Still, the real extent of non-coding RNAs contribution to Polycomb recruitment and function remains to be determined. Our project aims at identifying and characterizing all the non-coding RNAs involved in PcG-mediated gene regulation, and their specific role in the myogenic differentiation programme.

Using techniques relying on high-throughput technologies, such as CAGE, ChIP-seq and RIP-seq, we have generated a set of databases where dynamic changes in transcription profile, Polycomb binding sites and Polycomb-associated RNAs are analyzed genome-wide in C2C12 myoblasts and derived myotubes. The crosstalk between these different databases will eventually lead us to an integrated view of the molecular machineries that control the specific recruitment of PcG complexes on their chromatin target sites. Such a deeper understanding of both PcG and ncRNA functions in myogenesis regulation will be of great relevance to determine the molecular basis of muscle diseases in humans.

The methyltransferases CARM1 and PRMT5 control differentially myogenesis in zebrafish

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In vertebrates, skeletal myogenesis involves the sequential activation of myogenic factors to lead ultimately to the differentiation into slow and fast muscle fibers. How transcriptional co-regulators such as arginine methyltransferases PRMT4/CARM1 and PRMT5 control myogenesis in vivo remains poorly understood. In this study, we show a positive, but differential, role of these enzymes during myogenesis in vivo. While PRMT5 regulates myod, myf5 and myogenin expression and thereby slow and fast fiber formation, CARM1 regulates myogenin expression only, fast fiber formation and does not affect slow fiber formation. However, our results show that CARM1 is required for proper m-cadherin expression, thereby controlling slow fiber migration. Altogether, our results reveal that CARM1 and PRMT5 control differentially myogenesis in zebrafish embryos and identify CARM1 as a new player in slow fiber migration.

Reversible Immortalization of Human Mesoangioblasts: Towards Autologous Cell Therapy for Duchenne Muscular Dystrophy

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Human mesoangioblasts (hMABs) are vessel–associated progenitors able to differentiate into skeletal muscle and to reconstitute the damaged muscle fibers when injected in a mouse model of Duchenne muscular dystrophy (DMD), an incurable myopathy for which no therapy currently exists. Because of these features hMABs are now entering clinical experimentation based upon allogeneic transplantation for DMD. Unfortunately, upon long-term culture hMABs reduce proliferation and undergo senescence. Replicative senescence is an obstacle in the case of autologous cell therapy, where expansion is necessary, in particular for low-efficiency gene-correction strategies. In this context, the aim of this project is to reversibly immortalize hMABS to create cell lines with an unlimited proliferative potential that can be finely controlled. Here we overcome the problem of hMAB senescence by expressing the catalytic subunit of telomerase (hTERT) and polycomb group gene Bmi-1. To reversibly immortalize hMABs, we have utilized lentiviral vectors encoding floxed IRES-HSV1-TK hTERT and Bmi-1: in this way Cre recombinase will remove the transgene sequences from the cells genome, thus eliminating the risk of tumorigenesis. Moreover, cells that may have escaped Cre–mediated vector excision will be eventually eliminated with Gancyclovir. Three normal clones have been characterized for proliferation and proper expression of hTERT and Bmi-1; they also remained growth factor-dependent, contact-inhibited, not tumorigenic and myogenic. We are now immortalizing hMABs from different DMD patients: this will allow us to genetically-correct them even utilizing new promising tools such as transposons and human artificial chromosomes.

Inducible activation of Akt increases skeletal muscle mass and force without satellite cell activation

Bert Blaauw
Venetian Institute of Molecular Medicine

A better understanding of the signaling pathways that control muscle growth is required to identify appropriate countermeasures to prevent or reverse the loss of muscle mass and force induced by aging, disuse or neuromuscular diseases. However, two major issues in this field have not yet been fully addressed. The first concerns the pathways involved in leading to physiological changes in muscle size. Muscle hypertrophy based on perturbations of specific signaling pathways are either characterized by impaired force generation, e.g. myostatin knockout, or incompletely studied from the physiological point of view, e.g. IGF-1 over-expression. A second issue is whether satellite cell proliferation and incorporation into growing muscle fibers is required for a functional hypertrophy. To address these issues, we used an inducible transgenic model of muscle hypertrophy by short-term Akt activation in adult skeletal muscle. In this model, Akt activation for three weeks was followed by marked hypertrophy (approximately +50% of muscle mass) and by increased force generation, as determined in vivo by ankle plantar flexor stimulation, ex vivo in intact isolated diaphragm strips and in single skinned muscle fibers. No changes in fiber type distribution and resistance to fatigue were detectable. BrdU incorporation experiments showed that Akt-dependent muscle hypertrophy was accompanied by proliferation of interstitial cells but not by satellite cells activation and new myonuclei incorporation, pointing to an increase in myonuclear domain size. We can conclude that during a fast hypertrophic growth myonuclear domain can increase without compromising muscle performance.

Repetitive elements transcription and mobilization contribute to human skeletal muscle differentiation and Duchenne muscular dystrophy progression

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Noncoding RNAs (ncRNAs) are recently considered component of chromatin, having a critical role in organizing the epigenome architecture and epigenetic memory.
Genome-wide studies have revealed that ncRNAs transcription, mostly originating within intergenic regions of the genome, is far more ubiquitous than previously thought. A large part of thee transcripts originate from repetitive sequences. To this, we recently reported the first complete transcriptome produced by repetitive elements in the mammalian genome (Faulkner et al, Nat Genet 2009), which covers about 20% of overall transcripts in a cell. This study revealed that repetitive

element expression is regulated in a tissue specific manner and that their expression is positively correlated with expression of neighboring genes. Notably, LINE signal dependent expression appears to be linked to their genomic redistribution, as recent reports showed de novo LINE-1 (L1) retrotransposition events in somatic as well as cancer cells (Coufal et al., Nat 2009; Huang et al., Beck et al, Iskow et al. Cell 2010). It as also been shown that L1 retrotransposition can be controlled in a tissue-specific manner and that disease-related genetic mutations can influence the frequency of L1 retrotransposition (Muotri et al Nat 2010). These findings suggest a potential role of mobile elements as mediators of somatic variations, which in turn can influence the genome and the epigenome plasticity in order to accomplish developmental programs.
The role of noncoding transcriptome in skeletal muscle cell differentiation is unexplored and it may represent an opportunity to unravel and characterize its contribution to dystrophic muscle degeneration.

To this we generated deepseq transcriptome CAGE libraries from three Duchenne Muscular Dystrophy (DMD) patients and three controls' primary myoblasts. Cytosolic and nuclear RNA fractions were collected and deep-sequenced at different time points: proliferating myoblasts, myotubes upon differentiation induction (day 1 of differentiation) and differentiated myotubes (day 8 of differentiation). This analysis highlighted that LINEs constitute the bulk of repetitive element transcription and that the resulting RNAs are selectively localized in the nucleus. Notably the largest difference between DMD and control samples appears to be in nuclear transcriptome of all repetitive elements including LINE-1. Further, by using a Taqman-based approach, we analysed L1 copy number variation in proliferating and differentiating myoblasts derived from the same DMD patients and healthy donors; surprisingly, new retrotrasposition events occured during control's differentiation and not during DMD's differentiation.

Current efforts are aimed at establishing a direct link between L1 transcription, myogenic program and its alteration in DMD progression.

The maintenance of myogenic progenitor and satellite cells depends on a Notch- and Hes1-dependent suppression of MyoD

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Max-Delbrück-Center for Molecular Medicine

Skeletal muscle development, growth and regeneration rely on the maintenance of myogenic progenitor and stem cells. These cells are defined by the expression of Pax7 and Pax3, and they either self-renew or give rise to differentiating cells. Elimination or attenuation of canonical Notch signaling results in a premature depletion of the myogenic progenitor pool during development, and in the formation of tiny muscle groups. We show here that these drastic effects are rescued by the mutation of MyoD. Furthermore, we show that the direct Notch target gene Hes1 controls the maintenance of myogenic progenitors during embryonic development and of satellite cells in the postnatal phase by suppressing MyoD. In addition, we show that Hes1 is important for the self-renewal of adult satellite cells.

Necdin protect myoblast from programmed cell death by controlling specific protein degradation

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Necdin, a member of the MAGE family, is a key player in muscle regeneration. We have shown that Necdin is expressed in satellite cells and is able to sustain efficient muscle differentiation and regeneration by acting on two different pathways: on myoblast differentiation, by direct transcriptional regulation of myogenin, in cooperation with MyoD and by protecting myoblasts from cell death. We have shown that necdin inhibit the activation of both Caspase 3 and 9, probably by exerting its function via the intrinsic death pathway, but still the precise molecular mechanism is unknow.
By using the Two Hybrid system in yeast we were able to identify new interactors of Necdin in myogenic cells. In our study, we identified for the first time that Necdin interacts with CCAR1 in myogenic precursors. This protein also known as CARP1, cell cycle and apoptosis regulatory protein-1, is a perinuclear protein that mediates apoptosis signalling by diverse agents, leading to the activation of caspase 9 and 3, members of Jun N-terminal kinase (JNK) and p38 MAPK family of proteins. We show that Necdin is able to regulate CCAR1 protein levels by facilitating its degradation through the proteasome, thus counteracting CCAR1 proapoptotic activity.

Endothelium-derived DLL4 and PDGF-BB recruit committed skeletal myoblasts to a pericyte fate during myogenesis.

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Pericytes are vessel associated mural cells that form the smooth muscle layer of vessels; they express different markers (SMA, ALP,etc), none of which is unique to pericytes or constantly expressed. Endothelial cells recruit pericytes from the surrounding mesoderm progenitors through a PDGF-PDGFrB loop and Dll4 signaling.
In developing skeletal muscle, these progenitors include skeletal myoblasts. They can be induced to a pericyte phenotype by the endothelium, whose action can be replaced by exposure to PDGF-BB and DLL4. Under these conditions, purified skeletal myoblasts that would spontaneously differentiate, fail to differentiate, continue to proliferate and express pericyte markers. This occurs, at a lesser extent, also in post-natal myogenic cells. When co-cultured with endothelial cells in matrigel plugs, myoblasts adopt a perithelial position and a pericyte morphology in the newly formed vessel network. Inhibition of gamma secretase completely restores myogenesis. Furthermore Notch activation in MyoD expressing embryonic or fetal cells in vivo, phenocopies the Myf5/MyoD double null embryos, with the notable exception of Myf5 that continues to be expressed at the protein level in a large number of cells in the mesenchyme, some of which are vessel associated and co-express pericyte markers. Myf5 cannot activate myogenin and trigger myogenesis because Notch, activates Twist that inhibits Myf5 transcriptional activity. There is an indication that endothelial cells are able to recruit committed skeletal myoblasts for the formation and stabilization of vessel wall, suggesting that during skeletal muscle histogenesis, cells adopt a skeletal or a smooth muscle fate depending on signals secreted from neighboring cells.

DEFINING THE ROLE OF CYCLIN D3 IN SATELLITE CELL FUNCTION AND MUSCLE REGENERATION

Giulia De Luca, Roberta Ferretti, Eleonora Mezzaroma, Maurizia Caruso

In previous studies we have shown that cyclin D3 is highly induced during myoblast cell differentiation in vitro through mechanisms controlled by MyoD and pRb, two pivotal regulators of skeletal myogenesis. To assess the role of cyclin D3 in the control of muscle progenitor cell function, we used RNAi technology to knockdown cyclin D3 protein levels in vitro and cyclin D3 knockout approach in vivo.
The inhibition of endogenous cyclin D3 expression in satellite cell-derived C2 myoblasts resulted in reduced proliferation, premature expression of differentiation markers and impaired myotube formation, indicating that cyclin D3 critically controls the balance between myoblast proliferation and differentiation.
In muscles from wild-type mice, cyclin D3 displayed high expression during the first weeks of postnatal life but no expression in the adult. Notably, however, cyclin D3 was induced in adult muscle during the early phases of injury-induced regeneration. Therefore, cyclin D3 appears to be induced in vivo during early post-natal and regenerative myogenesis.
Cyclin D3-null tibialis anterior (TA) muscles collected from 2-mo-old mice showed fewer Pax7+ satellite cells and a decrease in myofiber size when compared with wild-type controls. Furthermore, many fewer myoblasts were generated in muscle dissociation cultures derived from cyclin D3-null mice than from wild-type mice. Time-course analyses of injury-induced regeneration in muscle lacking cyclin D3 are in progress. Preliminary results indicate that at three days post-injury, cyclin D3-null TA muscles show a reduced number of proliferating precursors when compared to wild-type controls.

DEFINING THE ROLE OF CYCLIN D3 IN SATELLITE CELL FUNCTION AND MUSCLE REGENERATION

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Differential vulnerability of fast and slow skeletal muscles of rats with heart failure: impact of oxidative stress, contractile properties, and myogenic mRNA markers expression

Raquel Santilone Bertaglia, Joyce Reissler, Francis Lopes, Fernanda Carani, Ana Angélica Fernandes, Marcia Gallacci, Maeli Dal-Pai-Silva, Robson Carvalho [2]
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Heart failure is characterized by limited exercise tolerance with skeletal muscle atrophy and shift toward fast muscle phenotype. Changes in reactive oxygen species (ROS), muscle function, and myogenic mRNA markers expression also contribute to the myopathy during this syndrome. In the present study, we investigated these parameters from both Heart Failure (HF) and Control (CT) groups in muscles with different fiber-type composition and function: fast phasic extensor digitorum longus (EDL) and slow postural soleus (SOL). Heart Failure was induced by a single injection of monocrotaline (30mg/kg); lipid hydroperoxide (LH) concentration, and glutathione peroxidase (GSH-Px) and superoxide dismutase (SOD) activities were determined biochemically; contractile properties and fatigue resistance tests were analyzed by myography; MyoD and Myogenin gene expression were quantified by RT-qPCR; Myosin Heavy Chain isoforms were determined by Sodium Dodecyl Sulphate Polyacrilamide Gel Electrophoresis; muscle fiber type frequency and cross sectional area (CSA) were analyzed by myofibrillar Adenosine Triphosphatase. HF SOL muscle showed an increasing in LH concentration, decreasing in SOD and GSH-Px activities, increasing in muscle contraction fall time, and lower Myogenin mRNA expression. HF EDL muscle showed only a decreasing in MyoD mRNA expression. These findings show that monocrotaline induced HF promoted more prominent phenotype changes in SOL, an oxidative slow-twitch muscle. Although further experiments are required to clearly define the precise mechanisms involved in the myopathy that occurs during heart failure, our results add to the understanding of the muscle-specific changes in this syndrome.

Alpha sarcoglycan is required for FGF-dependent myogenic progenitor cell proliferation in vitro and in vivo

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Mice null for α-sarcoglycan (Sgca-null) develop a progressive muscular dystrophy and serve as a model for human limb girdle muscular dystrophy 2D. Sgca-null myopathy is more severe than the model for Duchenne muscular dystrophy (mdx) and the opposite of what is seen in humans. In an attempt to understand the cellular basis of this severe muscular dystrophy, we cloned myogenic progenitor cells (mpc), the resident post-natal muscle progenitors from dystrophic and wt mice. Mpc from Sgca-null mice generated much smaller clones than mpc from wt or mdx dystrophic mice. Impaired proliferation of Sgca-null myogenic precursors was confirmed by single fiber analysis and this difference correlated with the Sgca expression during mpc proliferation. In the absence of dystrophin and associated proteins, which are only expressed

after differentiation, SGCA complexes with and stabilizes the FGF receptor 1. Ablation of Sgca leads to FGFr1from the membrane and mpc proliferation is impaired in response to bFGF. The low proliferation rate of Sgca-null mpc was rescued by transduction with Sgca-expressing lentiviral vectors. When transplanted in dystrophic muscle, Sgca-null mpc exhibited reduced homing and proliferation. The reduced proliferative ability of Sgca-null mpc explains the severity of this muscular dystrophy and also why wt donor progenitor cells engrafted efficiently and consequently ameliorated disease.

Role of miRNAs in Duchenne Muscular Dystrophy

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Duchenne Muscular Dystrophy (DMD) is caused by mutations in the dystrophin gene and is one of the most severe myopathies. Various therapeutic strategies have been developed in the last decade: among them exon skipping is a promising approach which allows the rescue of dystrophin synthesis through the production of a shorter but functional messenger RNA. When thinking of possible intervention on human, it is crucial to identify the most appropriate antisense sequences. We are now working for the optimization of this strategy, selecting the most active antisense molecules for the skipping of different exons.
Moreover, taking advantage of this strategy, we analized the role of miRNAs, fine modulator of gene expression, in DMD pathology. Among miRNAs up-regulated in DMD condition we found miR-31. We demonstrated that this miRNA represses dystrophin expression by targeting its 3'UTR. In human DMD myoblasts treated with exon skipping miR-31 inhibition increases dystrophin rescue. These results indicate that interfering with miR-31 activity can provide an ameliorating strategy for those DMD therapies aimed at efficiently recover dystrophin synthesis.
We also found that muscle-specific miRNAs (miR-1, miR-133 and miR-206) are released in the bloodstream of DMD patients as consequence of muscle degeneration and that their levels correlate with the severity of the disease. We propose that those miRNAs represent valuable biomarkers not only for the diagnosis of DMD onset and progression but also for monitoring the outcomes of therapeutic interventions on humans.

A mouse defined model to study fibre-type dynamics in skeletal muscle

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The absence of valid experimental and genetic tools to study in vivo fibre conversions and/or to manipulate and pursue fibre-type within a muscle have impeded to better understand the process of fibre-type switching in response to external or pathological stimuli.
Secondary silent and primary enhancers interact to regulate the expression of the mouse Troponin I genes. This interaction is crucial to precisely guarantee the appropriate timing, tissue and fibre specificity, and their quantitative expression (Guerrero et al, 2010). In our laboratory, based in these data, we have produced transgenic mice carrying the primary and secondary enhancer elements from mouse fTnI gene upstream to the green fluorescence reporter gene and driving the corresponding fluorescence to fast fibres respectively. Thus, fast fibres have GFP fluorescence. These animals are a credible model and an extraordinary tool to visualize and quantify fibre-type changes within a muscle. Currently, we have produced the third generation to obtain homozygous offspring.
Here, we present the fibre-type characterization of the transgenic animals that enables us to follow green fluorescence in fast fibres. We have studied Soleus, EDL, Gastronemius and Diaphragm muscles by confocal microscopy and western analysis. Based in our data, we can say that green fluorescence is visualized exclusively in fast fibres revealing the number of fast Type II fibres (green). The rest of the fibres are slow Type I fibres and do not present any fluorescence. In the future, using non-invasive fluorescence imaging technology in anesthetised living animals we will try to pursue fast fibres changes within a muscle in response to external or internal stimuli.

Microgenomic Analysis in Skeletal Muscle: Expression Signatures of Individual Fast and Slow Myofibers

Francesco Chemello [1], Camilla Bean [1], Pasqua Cancellara [2], Paolo Laveder [1], Carlo Reggiani [2], Gerolamo Lanfranchi [1]
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Skeletal muscle is a complex, versatile tissue composed of a variety of functionally diverse fiber types. Genome-wide expression profiling of skeletal muscle find a major limit in the complex composition of this organ that is composed by heterogeneous myofibers and other cell types. As a consequence, microarray experiments performed with mRNA extracted from whole muscles or biopsies produce only averaged information.
We have set up the methodology to carry out transcriptome analysis at the single fiber level (microgenomics). By applying this approach in the mouse, we have produced a first catalogue of genes expressed in slow-oxidative (type 1) and fast-glycolytic (type 2B) fibers. Individual fibers were obtained from soleus and EDL muscles and initially classified by myosin heavy chain isoform content. Gene expression profiling on DNA oligonucleotide microarrays showed that both qualitative and quantitative improvements were achieved, compared to results with standard muscle homogenate. First, myofibers profiles were virtually free from non-muscle transcriptional activity. Second, thousands of muscle-specific genes were identified, leading to a better definition of gene signatures in the two fiber types. Third, several fiber-specific metabolic and signaling pathways were detected, thus bringing the analysis of the differentially expressed genes from simple lists to genomic networks.
We are now extending our analysis to fiber types with intermediate characteristics. Since microgenomics in skeletal muscle makes possible to reach an unprecedented resolution level, it would be very interesting to apply this innovative approach on muscles undergoing plastic transitions to better understand the molecular mechanisms controlling myofibers development and specification.

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Role of Necdin in mesoangioblasts dependent skeletal muscle differentiation and regeneration

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Cell therapy holds great potential for the treatment of muscular dystrophies. Mesoangioblasts (MABs) are vessel-associated stem cells recently identified as ideal candidates in this regard because of their significant contribution to restoration of muscle structure and function in different muscular dystrophy models.
In order to dissect the molecular pathways that regulate the growth and the differentiation of these cells we focused our study on the role of Necdin, a MAGE protein expressed in both developing and adult skeletal muscle that plays a pivotal role

in muscle tissue, being required for proper myoblasts differentiation, for the first phase of muscle fibers growth and for efficient repair upon muscle injury. Moreover Necdin acts at different levels promoting myoblasts differentiation and survival.
We have isolated and characterized muscle-derived MABs from an adult wt mouse and we have demonstrated that Necdin enhances muscle differentiation and regeneration by these cells. Indeed, when Necdin is constitutively over-expressed it accelerates their differentiation and fusion in vitro and it increases their efficacy to restore dystrophic phenotype of a-sarcoglycan null mouse. Moreover, Necdin confers a enhanced survival ability when mesoangioblasts are exposed to several cytotoxic stimuli. Taken together, these data demonstrate the pivotal role of Necdin in muscle reconstitution.
Understanding more in detail the molecular mechanism of the effect of Necdin on MABs is the next crucial step towards the optimization of cell therapy with MABs or other stem cells.

Impaired expression of miR669a and miR669q activate skeletal myogenic program in Sgcb-null cardiac progenitor cells

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Stem and progenitor cells that reside in the post-natal heart are extensively studied as a potential therapeutic tool for cardiomyopathies; however, not much is known on the control of cardiac differentiation in resident progenitor cells. Recent works have highlighted an important role of miRNAs as regulators of cardiac and skeletal myogenesis. Here we isolated cardiac progenitors from neonatal beta-sarcoglycan (Sgcb) null mouse hearts affected by dilated cardiomyopathy. Unexpectedly, Sgcb-null cardiac progenitors spontaneously differentiate into skeletal muscle fibers both in vitro and when transplanted into regenerating muscles or infarcted hearts. This is due to the lack of a novel identified miRNA, miR669q, and the downregulation of miR669a. Although other miRNAs are known to promote myogenesis, to date only miR669a and miR669q act upstream of Myogenic Regulatory Factors by directly targeting MyoD 3'UTR. This finding opens a new scenario on the fate choice of mesoderm progenitors and makes the task of utilizing endogenous cardiac stem cells more complex for certain genetic diseases.

Transcriptional regulation of Myf5 expression in the branchial arches

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Trunk musculature is derived from somites whilst the musculature of the head arises from prechordal mesoderm, occipital somites and the branchial arches (BAs). Irrespective of its origin, skeletal myogenesis is controlled by the four myogenic regulatory factors (MRFs): Myf5, Mrf4, MyoD and Myogenin. Myf5 is the first MRF to be expressed in the BAs during development. We have previously identified five elements responsible for driving this expression: i) the mandibular arch element; ii) the hyoid arch element; iii) the negative arch element; iv) the proximal arch element; and v) the intronic arch element.

We have now started to unravel how these elements interact in order to establish and maintain the correct expression pattern during craniofacial development. We show that these do not operate as "classical" modular enhancers, and thus some are not able to drive expression in isolation, while removal of single enhancers does not only affect the expression pattern driven by the enhancer in isolation. These results indicate that these five enhancer elements do not act as individual modules and that the combined pattern of the individual enhancers does not recapitulate the entire pattern of Myf5. Furthermore, we show that these enhancers require each other in order to drive the full pattern, suggesting that a network model is more appropriate to explain the regulation of Myf5 in the developing head muscles.

Finally, we are using Chromatin Immunoprecipitation (ChIP) combined with qPCR to identify the upstream regulators of Myf5 in the BAs at different developmental stages.

Therapeutic Effect of Genetically Modified Muscle Progenitor Cells in Acute and Chronic Motor Neuron Injury

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Previous studies showed that administration of Neurotrophic factors (NTFs), is protective in models of motor neuron diseases, such as ALS. However, most of the clinical studies failed to show significant improvements probably because they could not deliver efficiently and continuously the NTFs into the lesion sites.
We have previously described the isolation from adult skeletal muscle of low adherence myogenic cells that can proliferate in cultures as floating aggregates named myospheres. Cloned myospheres derived muscle progenitor cells (MPCs) have been engineered to express NTFs. Each clone expressed a single NTF gene: BDNF, GDNF, VEGF or IGF-1 (MPCs-NTF). A combination of the conditioned media collected from cultures expressing all four NTFs markedly rescued motor neuron cells (line NSC34), after 48 hours of hypoxia or exposure to H202. Furthermore, inoculation of the combined MPCs-NTF cell populations, transplanted one day after sciatic nerve crush in rats, restored motor functions, compared to inoculation of MPCs expressing a single kind of NTF.
Preliminary experiments in a mouse model of ALS indicate that intramuscular injection of MPCs-NTF cell populations mixture that together expressed all four factors, delayed the disease onset and markedly increased the mice lifespan. Thus, our findings suggest that a combination of several NTFs can synergistically protect motor neurons and alleviate symptoms in chronic and acute models of motor neurons injuries.. The experiments indicate a simple procedure to efficiently and constantly deliver various combinations of TNFs using muscle progenitor cells as vectors.

Long-term endurance training modifies skeletal muscle phenotype and does not alter calcineurin (CaN) gene expression.

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São Paulo State University, Brazil

Skeletal muscle is a plastic tissue consisting of muscle fiber types that express different myosin heavy chain (MHC) isoforms. Although many researchers have studied the molecular mechanisms involved in skeletal muscle adaptation to chronic resistance training, muscle responses during long-term endurance training remain unclear. This study aimed to test the hypothesis that muscle fiber-type transition during long-term endurance training (ET) may be related to calcineurin (CaN) mRNA expression changes in skeletal muscle. We used 16 male Wistar rats (4 months, 300-400g) divided into two groups: trained (TR; n=8) and control animals (C; n=8). TR underwent an aerobic training program of swimming for 8 weeks (5 days/week). Training volume and intensity were progressive: 10 min without overloading (1st week), 20 min, 1% overload (2nd week), 25, 30, 35, and 40 min, 3% (3rd week), 45, 50, 55, and 60 min, 5% (4th week and 60 min, 5% (5th to 8th weeks). At the end of the experiment animals were weighed and sacrificed. Plantaris (PL) muscle was removed and processed for biochemical and molecular analyses. MHC isoforms were analyzed by SDS-PAGE electrophoresis; CaN mRNA gene expression was analyzed by RT-qPCR. ET decreased MHCIID and increased MHCIIA percentages and did not alter CaN mRNA expression in the studied muscle. Our data suggest that the PL muscle phenotype changes in TR can enhance the oxidative capacity of muscle during long duration events; CaN is not involved with skeletal muscle phenotype changes during long-term endurance training.
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ORIGIN AND SPECIFICATION OF MUSCLE SATELLITE CELLS

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Muscle satellite cells are the stem cells of skeletal muscle. The biology of satellite cells in the adult has been well studied, but their origin in the embryo remains unclear. Previous studies have indicated that satellite cells originate from the somites and in particular from a Pax3/7-positive population of cells located in the central region of the dermamyotome of the somite. But these studies did not indicate the origin of the satellite cells prior to somitogenesis, nor whether they come from the same or different region as the myoblasts that form the primary and secondary myofibers. Fate mapping at the open neural plate stage was carried out in Xenopus embryos using orthotopic grafts from transgenic donor embryo's expressing GFP. These studies showed that most satellite cells originate from the dorsolateral plate rather than from the paraxial mesoderm. Specification studies were made by isolation of explants from the paraxial and dorsolateral regions of neurulae and these also indicated that the satellite cell progenitors arise from the dorsolateral plate. In addition, we found that BMP induction in this region can increase generation of Pax7 positive cells in the tadpole tail. Conversely, the induction of Noggin in dorsolateral explants will block formation of satellite cells suggesting that BMP signaling is required in vivo for satellite cell formation. We conclude that, in contrast to the currently held belief of a paraxial mesoderm origin, satellite cell progenitors are initially specified in the dorsal part of the lateral plate mesoderm and later become incorporated into the myotomes. In addition, the initial specification occurs at the neurula stage and depends on the ventral to-dorsal BMP gradient in the early embryo.

Lineage tracing of muscle-derived stem cells based on Sca1 expression

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Skeletal muscle contains resident stem and progenitor cells that are able to differentiate into muscle fibers. There are a lot of controversies concerning the origin, potential and characteristic features of these cells. Two are the most common stem cell population accepted in the muscle field: the satellite cells and muscle derived stem cells (MDSC).
Satellite cells are unipotent stem cells, positive for Pax7, located between the sarcolemma and the basal lamina of the muscle fiber. They are quiescent until activated upon injury.
The second population, MDSC, consists of multipotent stem cells that are able to differentiate several lineages (e.g. muscle fibers, adipocytes). It is not clear the relation between MDSC and satellite cells. Their phenotype is not completely defined; only Sca-1 has been consistently identified and considered as their marker.
In this study we used a triple transgenic mouse to genetically lineage trace the fates of Sca1 derived cells in vivo during normal aging and muscle regeneration after cardiotoxin damage. The preliminary results support MDSC as a predecessor of satellite cells. To further support this observation, the fates of Scal-derived cells were followed in a disease model (mdx mice). To elucidate the mechanism in which our labeled cells contribute to the renewal of muscle fibers, the function of Pax7 was impaired specifically under the control of Sca1 using a Pax7 conditional allele. In this conference, we would like to share our preliminary results about the functions of Sca1-positive MDSC during aging and muscle regeneration as well as under pathological conditions.

Extracellular matrix remodeling accompanies axial muscle differentiation and morphogenesis in the mouse.

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Genetic hierarchies involved in muscle determination and differentiation have been intensely studied, but comparatively less is known about how these genetic hierarchies translate into alterations in cell morphology leading to the morphogenesis of muscle. The obvious candidates to support changes in cell shape and cell translocation are molecules of the extracellular matrix (ECM). In this study we used immunostaining in sections and whole mount 3D imaging to map the distribution of the ECM molecules laminin, fibronectin and tenascin from early stages of myotome development to the formation of early epaxial muscle masses in the mouse embryo.
First, we showed that laminin undergoes dynamic cycles of assembly and disassembly during myotome development, indicating that apart from regulating early myogenesis, laminin matrices may be instrumental in containing muscle cells and thus creating a microenvironment that guides them in their differentiation programme. Second, we found that both fibronectin and tenascin are highly enriched at intersegmental borders and that they are in a position to bind cells through their alphavbeta1/alphavbeta3 and alpha9beta1 integrins. Fibronectin and tenascin also invade the myotome, extending "fingers" of matrices primarily between the myotome and the dermomyotome that might be used by muscle precursor cells when they enter the myotome from the dermomyotome.

These results allow us to significantly improve our knowledge on the 3D distribution and potential relevance of the ECM during skeletal myogenesis.

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These results allow us to significantly improve our knowledge on the 3D distribution and potential relevance of the ECM during skeletal myogenesis.

Deregulation of Ku80 results in stem cell dysfunction and aging phenotypes in skeletal muscle and is partially rescued by loss of p53

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Skeletal muscle progenitors are required for growth, maintenance, and regeneration. Muscle progenitors express PW1, a p53-effector involved in stress signaling pathways and muscle atrophy. Loss-of-function of a key enzyme required for DNA double strand repair (Ku80) leads to chronic p53 activation coupled with reduced postnatal growth and premature senescence. We show that Ku80 mutant and heterozygous skeletal muscles exhibit characteristics of premature aging. At birth, mutant and heterozygote muscles had elevated levels of activated p53 concomitant with an overall decrease in cellularity and stem cells. Ku80 heterozygotes displayed impaired muscle regeneration whereas Ku80 mutant muscle was unable to regenerate only following repeated injury. Loss of Ku80 autonomously affects muscle progenitor self-renewal and differentiation. Loss of p53 rescues normal myofiber characteristics in Ku80 heterozygote muscles, however, normal regeneration is not rescued due to changes in the stem cell niche. In addition, we provide evidences that muscle stem cells respond in a different manner to stress signaling than fibroblasts.
Whereas complete loss of Ku80 leads to catastrophic aging found in progeric disorders, loss of a single Ku80 allele results in an aging phenotype in muscle that more closely reflects normal human aging.

CD34+ fetal muscle cells differentiate into distinct cell types that are required for efficient adult muscle regeneration

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We have previously demonstrated that fetal muscle-derived CD34+ cells have a remarkable ability to repair dystrophic mdx mice muscle. In the present work we used different cell surface markers CD31, CD45, Sca1 to test the capacity of CD34+ sub-populations to differentiate and to identify the subset with a high ability to regenerate skeletal muscle. We first carried out an immunohistological investigation using stem cell and phenotypic markers to document the tissue location of CD34+ cells. We took advantage of these surface stem cell markers for immunomagnetic-beads cell sorting. The different fractionated cell populations were quantified by flow cytometry analysis (FACS) and RT-qPCR analysis was carried out to determine their genetic profiles. The next step was to test the differentiation potential of CD34+ subfractions using in vitro culture and implantation into regenerating mouse muscle. We showed that CD34+ population comprised a cell subset CD34+/CD31+/CD45- exhibiting endothelial characteristics and capable to form microvessels in vivo. CD34+/CD31-/CD45-/Sca1+ subpopulation located in the muscle epimysium showed adipogenic differentiation both in vitro and in vivo. Most of the cells in the CD34+/CD31- fraction were CD45-/Sca1-, localized in the muscle interstitium, and transcribed myogenic genes. These cells were myogenic in vitro, and efficiently contributed to skeletal muscle regeneration in vivo, although their myogenic potential was lower than that of unfractionated CD34+ cells. Our results suggested that the other cell type grafted with myogenic cells enhance entry of progenitors into the myogenic program. Subfractionation of such defined muscle sub-population could be of great interest for cell therapy.

Understanding patterning during axolotl limb regeneration.

Eugeniu Nacu, Mareen Glausch, Febriyani Rochel Damanik, Maritta Schuez, Dunja Knapp, Elly Tanaka

We investigated which cell types are responsible for patterning the regenerating salamander limb. Cells of a salamander limb have positional identity – a molecular machinery that tells the cell its location in the limb. During regeneration cells can only acquire identities that are more distal (further away from the shoulder) of the amputation plane, what is termed the rule of distal transformation (RODT). We showed that muscle-derived blastema cells from the lower arm contributed to upper arm muscle regeneration, breaking the RODT. Muscle cells broke the RODT at the molecular level as well: they upregulated the transcription factor MEIS, an upper arm identity regulator, after upper and lower arm amputation. Connective tissue cells obeyed the RODT and also upregulated MEIS only upon upper arm amputation. Combined with previous knowledge, that Schwann cells have no positional identity, our results show that the connective tissues are responsible for patterning the limb during regeneration.
A second question we answered was how the positional identity machinery is activated during regeneration. We looked at activation in the upper arm and used MEIS as marker. We found that a tissue injury is not sufficient, and that the axons play a role in activating MEIS. By contrast, when we looked in the lower arm at activation of HoxA11, a marker of lower arm identity, we found that it was independent of the axons. These results indicate that different positional identity markers might be regulated differently during regeneration.

The histone methylase SMYD3 regulates satellite cells differentiation by modulating myostatin expression

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SMYD3 is a histone methylase that di- and tri-methylates histone H3 at lysine 4. In normal conditions SMYD3 is highly expressed only in the embryo, in adult skeletal muscle and in few other tissues but it is frequently upregulated in cancers; its transcriptional activity is involved in the proliferation of tumor cells.
In light of SMYD3 restricted expression in adult tissues and in different cell lines, we asked whether it might play a role during myogenesis and/or muscle maintenance. SMYD3 depletion in satellite cells and in C2C12 cells significantly accelerated myotubes formation and the differentiation process and was rescued by SMYD3 reintroduction by retroviral infection. Expression profiling of SMYD3 depleted C2C12 cells revealed increased expression of IGF1 and 2 and provided a set of putative SMYD3 target genes.
SMYD3 downregulation by ShRNA-interference resulted in decreased levels of myostatin transcript and protein both in satellite cells and C2C12 myoblasts, hinting for a direct regulation of myostatin transcription by the SMYD3 activator. Chromatin immunoprecipitation analysis (ChIP) revealed that SMYD3 is recruited to the promoter of the myostatin gene, and regulates histone H3K4 di- and tri-methlylation of myostatin regulatory regions. Furthermore, ChIP experiments revealed that SMYD3 modulates myostatin gene expression in the early steps of the elongation process.
Thus, our results identify SMYD3 as a new coactivator for myostatin transcription, providing a possible link between SMYD3 expression and muscle mass determination and homeostasis.

Role of Sphingosine-1-phosphate receptors in satellite cell function

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We have previously shown that the biolipid, sphingosine-1-phosphate, regulates muscle satellite cell activation (Nagata et al. 2006, JCB 174, 245-53). Here, we have examined the role of the receptors for sphingosine-1-phosphate in satellite cell function. We found that sphingosine-1-phosphate-receptor 3 (S1PR3) is highly expressed in quiescent satellite cells, but then levels significantly decrease as satellite cell activate and begin to proliferate. Retroviral-mediated constitutive expression of S1PR3 in proliferating satellite cells led to their exit from the cell cycle, with increased levels of the cell cycle inhibitors p21 and p27. However, the myogenic program was unaffected. By contrast, satellite cells from S1PR3-null mice show enhanced proliferation. They also differentiate to a greater degree, to form larger myotubes than control cells.
To examine the role of S1PR3 in vivo, we injured the tibialis anterior muscle with cardiotoxin and monitored regeneration. Seven days after injury, we found that regeneration was augmented in the S1PR3-null mice, with more regenerated myofibers per unit area, compared to heterozygous mice. However, by 3 weeks after injury, this difference was no longer observed. Challenging the muscle using multiple rounds of regeneration (1 injection of cardiotoxin per week for 3 weeks), we found that S1PR3-null muscle recovered better than control mice, when analysed a week after the final injection.
In conclusion, S1PR3 is involved in the maintenance of satellite cell quiescence and its absence leads to increased satellite cell proliferation. This increased proliferation presumably augments the efficiency of muscle regeneration.

Molecular regulation of muscle satellite cell via two Notch effector genes

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During postnatal development, satellite cells divide to provide new myonuclei to growing muscle fibers, and then change to an undifferentiated quiescent state in adult skeletal muscle. This state is considered a requisite for sustaining the satellite cell compartment, but the molecular mechanism is largely unknown.
In this study, we show that hesr1 and hesr3, Notch effector genes, are important regulators that are responsible for generation of undifferentiated quiescent satellite cells. Although both hesr1 and hesr3 single knock-out mice show no obvious abnormality in skeletal muscle, loss of both genes leads to increased expression of MyoD, myogenin, and Ki67 (a proliferation marker) in adult satellite cells. and a gradual decrease in the number of satellite cells with age. A gradual decrease of satellite cell pool and age-dependent regenerative defect were observed in hesr1/3 double knock-out mice. Our findings have implications for the roles of hesr family genes in the maintenance of tissue homeostasis and would facilitate the investigation of molecular regulation of satellite cells.

Role of the NAD+ Dependent Deacetylase SIRT1 in Skeletal Muscle Metabolism

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The NAD+-dependent deacetylase SIRT1 is a key energy sensor that regulates important metabolic processes in several organs, including liver and pancreas, however very little is known about SIRT1 metabolic functions in the skeletal muscle. To study the role of SIRT1 in skeletal muscle metabolism, we have generated SIRT1(i)skm-/- mice, in which SIRT1 has been selectively ablated in skeletal myofibers at adulthood. As SIRT1(i)skm-/- mice appeared metabolically unremarkable under basal conditions, we challenged them with high fat diet (HFD). After 12 weeks of HFD, SIRT1(i)skm-/- mice exhibited a significant decrease in their peripheral insulin sensitivity, when compared to control mice, as indicated by impaired blood glucose clearance during an insulin tolerance test. However, when the HFD was prolonged up to 20 weeks, SIRT1(i)skm-/- mice appeared as insulin sensitive as their controls, and more interestingly, after 32 weeks of HFD, SIRT1 (i)skm-/- mice became slightly more insulin sensitive than their controls. To gain insights into this apparent dual role of muscle SIRT1, we have performed gene expression analysis in the skeletal muscles of SIRT1(i)skm-/- and control mice, collected at different times of HFD. We have found that the reduced insulin sensitivity observed in SIRT1(i)skm-/- mice after 3 months of HFD is likely ascribable to reduced expression of genes in the insulin signaling pathway, while their improved insulin sensitivity (after 8 months of HFD) is the result of compensatory mechanisms, likely involving AMPK and PGC1-α activation and

increased expression of oxidative phosphorylation genes.
Taken together, our findings suggest that SIRT1 inactivation in skeletal muscles, by activation of compensatory mechanisms, may result beneficial in the treatment of metabolic disorders.

Haploinsufficiency of Myf5 reveals distinct cell fate potentials for adult skeletal muscle stem cells

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Satellite cells represent a heterogeneous population, and different markers identify subsets of cells. Skeletal muscle stem cell fate in adult mice is regulated by critical transcription factors, including the determination genes Myf5 and Myod. However, the precise role of Myf5 in regulating quiescent muscle stem cells has remained elusive.
We show that Myf5 protein is expressed in satellite cells. We found that heterozygous Myf5GFP-P/+ mice mark a subpopulation of muscle stem cells that are primed for myogenic commitment. We investigated by cell transplantation, and also after a second round of injury by notexin, the regenerative potential of Myf5GFP-P/+ and Tg:Pax7-nGFP satellite cells isolated by FACS from skeletal muscles of adult mice. Myf5GFP-P/+ and Tg:Pax7n-GFP satellite cells yielded equivalent numbers of regenerated PLAP+ fibers. However, a higher amount of GFP+ cells was found in a satellite cell position in the engrafted muscles from Myf5GFP-P/+ compared to Tg:Pax7nGFP mice. Consistent with this observation, muscle stem cells null for Myf5 exhibit a higher self-renewal capacity after transplantation compared to their wild-type counterparts.
Since Myf5GFP-P/+ cells represent a more primed cell population, our results suggest that Myf5 levels in muscle satellite cells modulate heterogeneity in behaviour and these levels influence cell fate decisions. These findings have important implications for how muscle stem cell fate is regulated, and they provide insights into understanding the heterogeneous nature of stem cell populations.

Analyzing the role of mouse Numb/Numblike in the adult muscle satellite cell pool

Nicole Gensch , Thomas Braun
Max-Planck-Institute for Heart and Lung Research

Satellite cells are the adult stem cells of the skeletal muscle. Different models are reported that explain self-renewal and differentiation of satellite cells in muscles. An evolutionarily conserved regulator implicated in cell fate determination in various developmental processes, including Drosophila myogenesis, is the plasma membrane-associated cytoplasmic protein, Numb. This endogenous Notch inhibitor was shown to be asymmetrically distributed during mitosis in the dermomyotome of mouse embryos as well as in dividing satellite cells where it potentially regulates the (stem) cell fate choice.
In this study we used the Cre/LoxP system to investigate a possible functional role of Numb and its related protein Numblike, during muscle satellite cell self-renewal. Conditional deletion of Numb (Numbflox/flox) in muscle progenitor cells on a Numblike-/- background showed a normal embryonic and postnatal skeletal muscle growth. Interestingly, loss of Numb in Myf5-derived muscle cells resulted in a decreased number of Pax7-positive satellite cells on flexor digitorum brevis (FDB) myofibers. We cultured intact FDB fiber and pulse-chased those with the BrdU analog, EdU, to examine satellite cell activation. The number of single EdU-positive satellite cells (Pax7-positive) was significantly reduced on fibers derived from Myf5:Cre+/Numbflox/flox/Numblike-/- mutant mice after 24-hour chase. Cell clusters of 2-4 cells resident on fibers showed no difference in the asymmetric distribution of Myf5 and/or Pax7 compared with cell clusters on WT fibers.
We report here that Numb/Numblike are not necessarily important for the development of the skeletal musculature. However, the decreased number of EdU and Pax7 double positive satellite cells on cultured fibers of Myf5:Cre+/Numbflox/flox/Numblike-/- mutant mice, suggest that Numb/Numblike are potentially involved in the early step of satellite cell activation (the G1 to S-phase transition in the cell cycle).

SHORT-TERM TREATMENT WITH CLEMBUTEROL SUPPRESSES PROTEASOMAL AND LYSOSOMAL PROTEOLYSIS AND ATROGENES EXPRESSION IN RAT DENERVATED SOLEUS MUSCLES INDEPENDENTLY OF AKT SIGNALING

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The present study was undertaken to clarify the mechanisms and signaling events involved in the reversal of denervation (DEN)-induced muscle atrophy by clenbuterol (CB), a selective β2-adrenergic agonist. Rats were submitted to sciatic nerve section (i.e, DEN) and rates of proteolytic systems, mRNA levels of atrogenes (atrogin-1, MuRF1, cathepsin-L) and autophagy markers (LC3b, GABARAPl1), IGF-1 content, and Ser473/Thr308-Akt phosphorylation levels were examined in soleus muscles 3 days after surgery. DEN reduced muscle mass (30%) and increased the lysosomal (110%) and Ub-proteasome (140%) proteolytic activities. Furthermore, DEN up-regulated atrogin-1 (~10-fold), MuRF1 (3.5-fold), cathepsin-L (54%), LC3b (2-fold) and GABARAPl1 (3-fold) mRNAs. The in vivo (3 mg/kg, 3 days, s.c.) treatment with CB concomitant with DEN attenuated the loss of muscle mass (50%) and the hyperactivation of the proteolytic systems. DEN-induced the up-regulation of atrogenes, but not autophagy markers, was significantly prevented by CB in vivo and in vitro (10-6 M CB, 2 hours). These effects were not associated with an increase either in IGF-1 content or in Ser473/Thr308-Akt phosphorylation levels. In isolated denervated soleus, triciribine (10μM), an Akt inhibitor, was unable to block the inhibitory effects of CB on proteolysis and atrogenes expression but 6-BNZ-cAMP (500μM), a PKA activator, mimicked CB effects. These data indicate that short-term treatment with CB mitigates DEN-induced soleus atrophy through a down-regulation of atrogenes and consequent inhibition of lysosomal and proteasomal activities, being these effects independent of Akt, and possibly mediated by PKA.
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Adiponectin expression in skeletal muscle cells in response to hypertrophic stimuli

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Adiponectin, one of adipocytokines, has insulin-sensitizing effects. Exercise increases in plasma level of adiponectin, might be secreted by adipose tissue. Recently, several reports showed that adiponectin protein expresses in skeletal muscle cells. Therefore, a part of plasma adiponectin might be derived from skeletal muscle cells. In the present study, we examined the changes in adiponectin expression in skeletal muscles in responsible to hypetrophic stimuli. The expression of adiponectin mRNA was evaluated by q-RT-PCR in both growth and differentiation phases of mouse myoblast cells (C2C12). The expression of adiponectin in mouse soleus muscles (C57BL/6J) also examined. Functional overloading on left soleus was induced by tenotomy for 4 weeks.
In C2C12, the expression of adiponectin mRNA was up-regulated in the differentiation phase. Increment in the protein and mRNA of adiponection in soleus muscles was observed following tenotomy. Results from this study indicated that the trained skeletal muscles might autocrine adiponectin. This study was supported, in part, by Grant-in-Aid for Scientific Research (B, 20300218, KG; A, 22240071, TY; S, 19100009, YO) from Japan Society for the Promotion of Science, and Mutual Aid Corporation for Private Schools of Japan (KG).

Dynamics of Myogenic Precursors in the Rat Hindlimb – A Role for Innervation

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This study investigates the dynamics of Pax3 and Pax7 +ve myogenic precursors as they migrate into the rat hindlimb, proliferate and differentiate, over the period from E12.5 – E16.5. It then correlates these processes with the timing of nerve

entry into the limb, and investigates the effect of limb denervation in utero. We found that Pax3 +ve precursors were present in the hindlimb from E12.5, whereas Pax7 +ve cells appeared at E13.5, concurrent with the entry of the limb innervation. Pax7 +ve cells were first seen in the central region of the limb, surrounding the nerves as they entered and branched within the limb; they then rapidly appeared within the Pax3 +ve muscle masses. Pax3 +ve cells proliferated at a steady rate (about 30% of cells labelled by a 1 hr exposure to BrDU) throughout development, until disappearing from the limb at E16.0. In contrast, proliferation of Pax7 +ve cells was initially steady (~30%), then accelerated to over 45% at E16.0 & E16.5. Denervation of the limb by injection of beta-bungarotoxin at either E15.5 or E16.5, followed by examination of EDL and TA muscle one day later, revealed a decrease in the number of Pax7 +ve progenitors in the muscles compared to untreated contralateral muscles. Concurrently we saw an increase in the number of myogenin +ve cells and a decrease in proliferation rate of Pax7 +ve cells (the latter significant only after injection at E15.5). From these results, we conclude that Pax7 +ve precursors in the limb are always in close physical proximity to the limb innervation and that balance between proliferation and differentiation in this population is profoundly dependent on the nerve.

Reg1 gene is expressed in regenerating muscle

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Introduction: Regeneration process can be induced in various tissues including skeletal muscles. Significant role during regeneration process is given to the Reg gene family including RegI gene. The aim of our study is to analyze possible RegI gene expression in regenerating rat skeletal muscle and corresponding nerve.
Methods: In 2-month-old Wistar rats, the regeneration process was induced in both type of muscles, slow (m.soleus, SOL) and fast (m. extensor digitorum longus, EDL). Muscle regeneration was induced by injection of 1 ml of local anesthetic. The RT-PCR analysis was performed for detection of RegI mRNA in regenerating muscles and sciatic nerves 6, 12, and 24 hours after regeration was induced.
Results. RegI gene is expressed in both types of skeletal muscles 6, 12 and 24 hours after regeneration process was induced. In both muscles, the peak expression of RegI gene is 12 hours after induction of regeneration, followed by decline 24 hours after regeneration process was started. In sciatic nerve that innervates the regenerating muscles, appearance of RegI mRNA occurs 12 hours and declines 24 hours after regeneration was induced.
Conclusions. For the first time we demonstrated the presence of RegI gene in regenerating skeletal muscle and sciatic nerve that innervates the regenerating muscles. Its expression is up regulated with regeneration process. Reg1 gene expression was induced in both skeletal muscle and nerve at a considerably early time point after muscle injury. Our results imply that Reg I gene has a significant role at the early stage of skeletal muscle regeneration.

Regulation of slow troponin c gene expression in the zebrafish

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The dissection of the molecular genetic pathways that specify different muscle fibre types is facilitated by their discrete temporal and spatial separation in the zebrafish embryo. Previous studies from our lab have identified the transcription factors Prdm1a and Sox6 as key regulators of muscle fibre type identity in this species. In the absence of Prdm1a function, fast twitch lineage specific genes are expressed ectopically in slow twitch progenitors, whereas loss of function of Sox6 leads to the ectopic expression of slow twitch genes in fast twitch progenitors. One such gene encodes the slow-twitch isoform of Troponin c. In contrast to mammals, which have a single slow troponin c (tnnc) gene that is expressed in both slow-skeletal and cardiac muscle, zebrafish has two paralogous genes, tnnc1a and tnnc1b, which are expressed specifically in the heart and slow-twitch skeletal muscle respectively (Sogah et al., 2010).
Using BAC recombineering we have generated transgenic lines that recapitulate the pattern of the endogenous tnnc1b gene in zebrafish embryos. We are using deletion analysis of this construct to identify the minimal regions required for this expression and in parallel using comparative sequence analysis to search for conserved regulatory regions in the first intron, where the human skeletal muscle enhancer has been shown to reside. Potential Sox6 binding sites have been identified and their functional relevance is being tested by site directed mutagenesis and chromatin immunoprecipitation (ChIP) using an anti-ZFSox6 antibody.

Multiple congenital malformations of Wolf-Hirschhorn syndrome are recapitulated in Fgfrl1 null mice

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Wolf-Hirschhorn syndrome (WHS) is caused by deletions in the short arm of chromosome 4 (4p) and occurs in about one per 20,000 births. Patients with WHS display a set of highly variable characteristics including craniofacial dysgenesis, mental retardation, short stature, speech problems, congenital heart defects as well as congenital diaphragmic hernia. Analysis of patients with 4p deletions has identified two WHS critical regions (WHSCRs); however microdeletions of these regions alone result in relatively mild phenotypes indicating that genes outside of the WHSCRs, that have not been conclusively identified, significantly contribute to the WHS phenotype. Recently, the human FGFRL1 gene, encoding a putative fibroblast growth factor (FGF) decoy receptor, has been implicated in the craniofacial phenotype of a WHS patient. Recently, we reported that targeted deletion of the mouse Fgfrl1 gene recapitulates a broad array of WHS-linked phenotypes, including abnormal craniofacial development, axial and appendicular skeletal anomalies, congenital heart defects and a prominent diaphragm defect (Catela C. et al, (2009) Dis. Model. Mech 2, 283–294). Despite a severe reduction in diaphragm size, which is the primary cause of death in newborn animals, FGFRL1 deficiency does not appear to affect other skeletal muscles during embryonic development. A focal point for current research is to dissect FGFRL1 role in diaphragm development and juxtapose it to the development and postnatal growth of other skeletal muscles. Taken together, these data support a wider role for Fgfrl1 in bone and muscle development, implicate FGFRL1 insufficiency in WHS and provide a novel animal model to dissect the complex aetiology of this human disease.

Expression of transcription factor TWIST during muscle development

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Skeletal myogenesis is a complex and tightly regulated process which involves the activation of a cascade of transcription factors. Myogenesis occurs during embryogenesis in order to form muscle and in adults to replace lost or damaged muscle. Most embryonic skeletal myogenic progenitors arise from somites, masses of paraxial mesoderm, which among to other tissues give rise to musculature. Twist is an important basic helix-loop-helix transcription factor expressed during early embryonic development. Certain experiments have been performed, mainly by overexpressing Twist in muscle cells, which indicate an inhibitory role in differentiation. No strong evidence exists regarding the expression and role of the endogenous Twist during muscle development. The purpose of this work was to investigate the expression of human TWIST in muscle cells isolated from various stages of foetal development. TWIST was found to be endogenously expressed in human foetal myoblast cell lines and its expression decreased during the late stages of development. Isolated human myoblasts showed an increasing capacity to differentiate into mature myotubes during development, accompanied by a decrease in the expression of myogenic regulatory factors (MRFs), such as MyoD. This implies an inversely proportional relation of TWIST expression with the differentiation capacity of isolated myoblasts. In conclusion, TWIST seems to play an important role in regulation of myogenesis during development and the mechanism by which this occurs is currently under investigation.

R- and P-cadherin as new targets for PAX3/7-FOXO1A fusion transcription factors: a role in RMS development?

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Rhabdomyosarcomas (RMS) are the most common soft tissue sarcomas of childhood. These tumours express skeletal muscle markers but are blocked into the differentiation program. Alveolar RMS (ARMS), which is the more aggressive subtype, is characterized by the expression of PAX3-FOXO1A or PAX7-FOXO1A fusion transcription factors. Although these factors have been clearly associated with ARMS etiology, little is known about their direct transcriptional targets that could explain their oncogenicity. Understanding the molecular and cellular mechanisms underlying ARMS pathogenesis would allow the identification of effective therapeutic targets and strategies.
We have identified in RMS a switch of cadherins characterized by a loss of N- and M-cadherin expression replaced by R-cadherin (R-cad), which is normally absent from adult muscles. Independent studies also demonstrated that P-cadherin (P-

cad) is strongly associated with ARMS tumours. Cadherins are adhesive transmembrane receptors known to mediate proliferation, differentiation, migration and invasiveness. Our data show that R- and P-cad co-localize with Pax3/7 muscle progenitor cells during embryogenesis and suggest that Pax3 acts genetically upstream of these cadherins. Moreover, i/R- and P- cadherin proximal promoters are activated by PAX-FOXO1A; ii/overexpression of PAX-FOXO1A in RMS cell lines leads to up-regulation of endogenous R- and P-cad and iii/PAX3-FOXO1A specifically binds the P-cadherin proximal promoter. We propose that through direct regulation of R- and/or P-cad, PAX3-FOXO1A could control major properties of ARMS cells such as proliferation and invasion. Targeting these cadherins may constitute an attractive therapeutic strategy against RMS.

Arp3 is essential for skeletal muscle formation and myoblast fusion in the mouse embryo.

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The Arp2/3 complex is a unique actin nucleator promoting the formation of branched actin polymers. The complex is necessary for normal cellular functions, such as migration, endocytosis, assembly of epithelial junction and others. Genetic investigations in Drosophila demonstrated a role of Arp3 in myoblast fusion.

To study Arp3 function during murine myogenesis we generated a conditional Arp3 mutant allele and utilized Lbx1-Cre mediated recombination to delete Arp3 specifically in muscle progenitors cells in limb buds.

Arp3 disruption in migrating muscle precursors resulted in drastically smaller hypaxial muscles in limbs and in a thinner diaphragm compared to control. These observations suggest that Arp3 is required for normal growth of skeletal muscle and/or survival of myogenic cells. Moreover, remaining muscle anlagen in homozygous Arp3del/del mutants revealed a reduction of multinucleated myotubes and a relative increase of mononucleated myoblasts. These myoblasts fail to align to typical facial arrays and therefore appear disorganized. Statistically significant reduced cell fusion was quantitated in cultured myocytes from mutant compared to control mouse embryos indicating that Arp3 is required for myotube formation, similar to its demonstrated function in Drosophila myogenesis.

Glucocorticoid receptor signalling is required for muscle atrophy caused by acute inflammation

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Skeletal muscle atrophy accompanies acute inflammatory conditions including ARDS, COPD exacerbations and sepsis, and closely correlates with increased mortality. Increased circulating glucocorticoid levels have been linked to muscle atrophy. Nevertheless, the contribution of glucocorticoid receptor (GR) signalling to muscle atrophy caused by acute inflammation remains to be elucidated. To investigate this, acute pulmonary inflammation was induced by intra-tracheal lipopolysaccharide (IT-LPS) installation, in muscle-specific GR knockout (MLC-Cre+/– GR-LoxP) and control (GR-LoxP) mice. Circulating cytokine and corticosterone levels were increased within 24h in response to IT-LPS. Increased expression of mRNA transcripts encoding glutamine synthetase and phosphoenolpyruvate carboxykinase, as well as Atrogin-1 and MuRF1, was detected in skeletal muscle of control mice following IT-LPS, suggestive of GR- and atrophy signalling. Importantly, the reduction in body and muscle weight following IT-LPS observed in GR-LoxP control mice, was strongly attenuated in muscle-specific GR knockout mice. These data implicate an important role for muscle GR-signaling in muscle atrophy following acute (pulmonary) inflammation.

β3-integrin mediates satellite cell differentiation in regenerating mouse muscle

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Skeletal muscle satellite cells can sense various forms of environmental cues and initiate coordinated signaling that activates myogenesis. Although this process involves cellular membrane receptor integrins, the role of integrins in myogenesis is not well defined. Here, we report a regulatory role of β3-integrin, which was previously thought not expressed in muscle, in the initiation of satellite cell differentiation. Undetected in normal muscle, β3-integrin expression in mouse hindlimb muscles is

induced dramatically from 1 to 3 days after injury by cardiotoxin. The source of β3-integrin expression is found to be activated satellite cells. Proliferating C2C12 myoblasts also express β3-integrin, which is further upregulated transiently upon differentiation. Knockdown of β3-integrin expression attenuates Rac1 activity, impairs myogenic gene expression and disrupts focal adhesion formation and actin organization, resulting in impaired myoblast migration and myotube formation. Conversely, expression of constitutively active Rac1 rescues myotube formation. In addition, a β3-integrin-neutralizing antibody similarly blocked myotube formation. Comparing with wild-type littermates, myogenic gene expression and muscle regeneration in cardiotoxin-injured β3-integrin null mice are impaired as indicated by depressed expression of myogenic markers and morphological disparities. Thus, β3-integrin is a mediator of satellite cell differentiation in regenerating muscle.

The intermediate filament protein Nestin regulates Cdk5 during myogenic differentiation

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Nestin is an intermediate filament (IF) protein expressed in many immature cell types, principally in neuronal and muscle cells. During differentiation, nestin is lost and replaced by other differentiation state-specific IF proteins. It is known that nestin regulates several important cellular functions, such as cell survival (Bieberich et al., 2003, Sahlgren et al., 2006) and motility (Kleeberger et al.,2007). Even though nestin is expressed during differentiation and regeneration of muscle tissue, its function in this particular context has not been properly addressed.
Cyclin-dependent kinase 5 (Cdk5) is a non-mitotic kinase that is activated by the non-cyclin proteins p35 and its stable cleavage product p25. Cdk5 is an important regulator of myogenic differentiation (Lazaro et al., 1997) as well as an organizer of NMJ:s (Fu et al., 2001). Deregulation of its activity is associated with apoptosis and several common neurodegenerative disorders. It was shown by Sahlgren et al. (2003, 2006) that nestin forms a regulatory scaffold for the Cdk5/p35 signaling complex in myoblasts and in apoptotic neuronal precursors. Recently our laboratory showed that PKCζ is a major regulator of muscle differentiation and acts upstream of Cdk5 by controlling p35 cleavage (deThonel et al., 2010).
My current studies are focused on how nestin contributes to muscle differentiation and the regulation of Cdk5. We have shown that nestin affects the onset and pace of differentiation. In addition, nestin and Cdk5 work in the same pathway regulating the pace of differentiation. Thus, nestin influences the myogenic outcome by regulating several signaling events during differentiation.

Down-regulation of Myogenin in terminal differentiated myotubes can reverse their differentiation.

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Certain higher vertebrates developed the ability to reverse muscle cell differentiation (dedifferentiation) as an additional mechanism to regenerate muscle. Mammals, on the other hand, rely only on the irreversible differentiation of mononucleated myoblasts into multinucleated myotubes. Myogenic Regulatory Factors (MRFs), MyoD, Myogenin, Myf5 and Myf6 are basic-helix-loop-helix (bHLH) transcription factors essential towards the regulation of myogenesis.
Our current interest is to investigate in detail whether down-regulation of MRFs in terminally differentiated myotubes can induce reversal of muscle cell differentiation.
Results in our laboratory show that Myogenin plays a prime part in the reversal of muscle cell differentiation. Down-regulation of Myogenin in terminally differentiated myotubes induces their cleavage into mononucleated cells. These mononucleated cells initiate DNA synthesis and express high levels of CyclinD1 and CyclinE2.
Finally, our results show that cleavage of myotubes following down-regulation of Myogenin occurs in the absence of growth factors and cell cycle.
In conclusion, down-regulation of Myogenin in terminally differentiated myotubes induces a reverse pathway for muscle cell differentiation followed by characteristic cellular changes which do not need the support of growth factors. These data reveal a novel mechanism by which muscle cells could be re-activated and also prove the vital role of Myogenin in maintaining muscle cell differentiation.

Requirement for signal dependent SRF/MEF2 network co-operativity for muscle specific enhancer activation

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An informatics approach to predict transcription factor interactions using hidden Markov models in which co-enriched cis elements in muscle specific enhancer/promoter regions were identified, revealed a strong enrichment between SRE (CArG) and MEF2 cis elements. Mutational analysis of multiple muscle promoters revealed an absolute requirement for both cis elements for robust transcriptional activation in muscle cells. Biochemical analysis revealed a previously unrecognized protein interaction between the SRF and MEF2 transcription factors. This protein interaction is co-ordinated by multiple common co-activators and repressors in a muscle specific interactome. The dynamics of these interactions were probed at muscle specific gene loci using chromatin immunprecipitation assays and this analysis indicated that productive temporal activation of the muscle creatine kinase enhancer during myogenesis is absolutely dependent on the co-operative recruitment of both the MEF2 (early) and SRF (late) components. Moreover, this network is dependent on the recruitment of the p38 MAP kinase and is repressed by PKA, protein phospatase 1 α and HDAC4. These data reveal a precise ordered pattern of recruitment of SRF and MEF2 to various muscle specific promoter/enhancers that determines a mechanism for the temporal pattern of transcriptional activation of various differentiation specific genes. Supported by CIHR

The muscle phenotype of the Compact mouse: myostatin and androgen effect?

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Selection for high protein content and body weight in the Technical University of Berlin resulted in a mouse strain, which showed a hypermuscular character similar to the KO mutant of myostatin, the growth inhibitor of skeletal muscle. The genetical mapping of the Hungarian subpopulation of the Compact strain revealed that the phenotype was not entirely monogenic. Beside the mutation in the myostatin propeptide, several loci e.g. androgen receptor gene region were proposed to modulate the Compact phenotype. So far, no data have proven the effect of androgens, however, a glycolytic hyperplastic muscle phenotype with only a moderate hypertrophy was described for Compact mouse.
Our aim was to investigate the fiber size and -distribution of the Compact hindlimb muscles by comparing the characteristics of male and female ones. Both histochemistry and myosin heavy chain immunohistochemistry were used for the analysis. In contrast to the results with the control Castaneus strain we detected an increase in both number and size of fast glycolytic IIB fibers in both male and female Compact muscles. However, males performed significantly stronger IIB fiber hypertrophy than females. A similar selective fiber hypertrophy has been described in response to testosterone treatment of the androgen-dependent levator ani muscle of castrated rats. These results suggest that androgen signaling is likely to influence the muscle phenotype of Compact mice.
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PW1 regulates mesoangioblast properties and identity

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Mesoangioblasts (MABs) are stem/progenitor cells associated with small vessels of mammalian mesoderm. In the last years, we have characterized MABs from embryonic and adult tissues, including mouse, dog and human. Although they cannot be identified by a unique marker and do not share the same gene expression profile, they have in common the ability to proliferate in culture, to undergo skeletal muscle differentiation and, most-importantly, to cross the vessel wall upon intra-arterial administration. Moreover, these cells have been successfully used in cell transplantation protocols, causing a significant rescue of structure and function in skeletal muscle of dystrophic mice and dogs. Interestingly, PW1 is expressed at high levels in murine, canine and human MABs. PW1 is a regulatory factor involved in cell stress response and participates in the regulation of signaling pathways controlling cell survival and death. Its role has been studied by loss of function experiments using PW1 shRNA in adult mouse MABs. We observed that silencing of PW1 dramatically inhibits the ability of MABs to differentiate in vitro through a mechanisms that leads to MyoD degradation. Moreover, in vivo experiments

showed that shPW1-MABs are not able to cross the vessel wall and migrate into myofibers, providing evidence that lack of PW1 in MABs may cause a defective ability of these cells to engraft in degenerating muscles via the arterial circulation. Since lack of PW1 in MABs interferes with the two key hallmarks of MABs, we conclude that PW1 is essential in conferring identity and the proper potency to MABs.

Proline isomerase Pin1 represses terminal differentiation and myocyte enhancer factor 2C function in skeletal muscle cells

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MEF2 (myocyte enhancer factor 2) proteins interact with members of the MyoD family of basic helix–loop–helix (bHLH) proteins to establish a unique transcriptional code for skeletal muscle gene activation. Recent studies have revealed multiple signaling systems that stimulate and inhibit myogenesis by altering MEF2 phosphorylation and its association with other transcriptional cofactors. We show that the Pin1 isomerase, which catalyzes the isomerization of phosphorylated Ser/Thr-Pro peptide bonds, interacts with phosphorylated MEF2C in muscle cells. This interaction requires two novel phospho-Ser-Pro motifs in MEF2C: Ser(98) and Ser(110), which are phosphorylated in vivo. Overexpression of Pin1 decreases MEF2C stability and activity and its ability to cooperate with MyoD to activate myogenesis. Furthermore Pin1 modulates the skeletal muscle differentiation program because down-regulation of Pin1 markedly promotes myogenic differentiation. We suggest that Pin1 is a novel regulator of MEF2C function and muscle differentiation, it is expressed in muscle cells and a significant proportion of Pin1 in myotubes but not in myoblasts is excluded from the nucleus. We observed a reduction of phosphorylation of the Ser(98) and Ser(110) Pin1 binding sites in differentiated myocytes. Based on these results we propose a model in which, in proliferating myoblasts, Pin1, upon binding to phosphorylated nuclear MEF2C, leads to decreased levels and transcriptional activity of MEF2C. Upon induction of terminal differentiation, to establish a full activity of MEF2 proteins, a reduced Pin1-MEF2C association is required, possibly due to the relegation of Pin1 to the cytoplasm and to a reduced level of phosphorylation of Ser98 and Ser110.

PAK1 and CtBP1 participate in the coupling of neuronal activity to muscle chromatin and gene expression.

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Gene expression in muscle is controlled by innervation, via both synaptic neural factors that activate synapse-specific gene expression, and neuron-induced electrical activity that represses myogenin expression. We show that in innervated muscle, the co-repressor CtBP1 is present on the myogenin promoter, together with the repressive histone mark dimethylated H3K9, hypo-methylated H3K4 and hypo-acetylated H3K9. CtBP1 inactivation is sufficient to de-repress myogenin expression in innervated muscle, and myogenin activation upon denervation coincides with CtBP1 removal from the promoter and reduction of H3K9 dimethylation as well as increased H3K4 methylation and H3K9 acetylation. CtBP1 removal from the myogenin promoter is link with its cytoplasmic translocation, a step which requires phosphorylation by PAK1. We further show that PAK1, whose expression is strongly activated after denervation, and which is required for acetylcholine receptor (AChR) synaptic clustering, accumulates at the NMJ, while CtBP1 is excluded from the subsynaptic nuclei. In cultured myotubes, CtBP1 is found on the promoter of the repressed AChRε gene. AChRε activation by neural factors is associated with CtBP1 removal and cytoplasmic relocalization. These findings reveal a molecular mechanism to account for the coordinated control of post-synaptic receptors aggregation, chromatin modifications and muscle gene expression by presynaptic neurons via PAK1 and CtBP1.

Switching of BAF SWI/SNF subunits regulates myogenic differentiation.

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Rhadomyosarcoma (RMS) is the most common soft tissue sarcoma of childhood. RMS cells resemble fetal myoblasts but are unable to complete the differentiation program. We have recently shown that the myomiR-206, which is not expressed in RMS, when reintroduced in RMS cells promotes the resumption of myogenic differentiation. We have now evidence that the ability of miR-206 to re-activate the myogenic program in RMS cells depends on its effect on the composition of the SWI/SNF chromatin remodeling complex. Gene expression profiling on miR-206-converted RMS cells revealed, among the downmodulated genes, the BAF53A subunit. We verified that BAF53A is a direct target of miR-206 and we found that BAF53A downregulation also occurs in C2C12 cells at the onset of differentiation. Interestingly, BAF53A down-modulation is associated to BAF60C up-regulation in both cells types. Conditional silencing of BAF53A in RMS cells is sufficient to promote BAF60C up-regulation and to activate the myogenic program. Conversely, over-expression of BAF53A (or silencing of the BAF60C subunit) strongly impairs differentiation, while overexpression of BAF60C alone is not sufficient to promote differentiation. Our data suggest that SWI/SNF complexes containing the BAF53A subunit have a repressive role on myogenic genes such as BAF60C and myogenin, and that downregulation of the BAF53A subunit by microRNAs is a necessary step to promote the transition to an activatory chromatin remodeling complex in myogenic loci.

Fibro/adipogenic progenitors, inflammatory cells and skeletal muscle fibrosis

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Tissue damage triggers various intracellular and intercellular pathways that restore tissue integrity and homeostasis either by regeneration or repair. Regeneration ensures the cellular integrity and functionality of the damaged tissue by replacing necrotic tissue with healthy activating resident stem or progenitor cells. In contrast, repair results in non-functional fibrotic mass, commonly referred to as scar tissue. Even though repair ensures tissue integrity, dysfunctional healing by scar formation compromises tissue function in aging and inflammatory pathologies.
We have recently identified a population of fibro/adipogenic progenitors (FAPs) residing in skeletal muscle and multiple other tissues. These cells respond to acute damage by entering the cell cycle earlier than the satellite cells and expanding to invade the space between the regenerating myofibres. During this phase, FAPs produce trophic factors that affect myogenic cell differentiation and enhance regeneration. If regeneration proceeds efficiently, FAPs are ablated from the environment by apoptosis and return to their initial numbers. While these cells normally exert a positive effect, they are recruited immediately following the failure of regeneration to generate fibrosis by differentiating into myofibroblasts and adipocytes. Interestingly, we observed that this complex interaction between myogenic and fibro/adipogenic cells is further modulated by inflammatory cells and cytokines. The results from this study, describing the role of inflammatory mediators, in modulating the Janus-like actions of FAPs will be presented at the meeting.

Maged1, a new regulator of skeletal myogenic differentiation and muscle regeneration

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Maged1 is a member of the large family of MAGE proteins that were initially identified as tumour antigens. The molecular and physiological functions of the MAGE proteins remain largely to be clarified, but they act probably as adaptor or scaffolding proteins able to modulate the function of transcription factors and membrane receptors. In normal adult skeletal muscle, cell turnover is very slow. However, after an acute lesion or in chronic pathological conditions, such as primary myopathies, muscle stem cells, called satellite cells, are induced to proliferate, exit definitively from the cell cycle and fuse to form new muscle fibres. The work presented here unveils the importance of Maged1 in myogenic differentiation and muscle regeneration. We show that Maged1 is expressed at very low levels in normal adult muscle but is strongly induced after injury, during the early phases of myoblast differentiation. By analyzing primary myoblasts derived from Maged1 knockout mice, we show that Maged1 deficiency results in reduced levels of p21CIP1/WAF1, Adam12 and Itgb1, leading to defective cell cycle exit and impaired myotube maturation. Our data suggest that Maged1 regulates the transcriptional activity of the key muscle transcription factors MyoD and Mef2C, explaining the myogenic differentiation failure. In vivo, we show that Maged1 deficiency results in delayed regeneration of muscles injured experimentally, confirming the physiological role of Maged1 in this process.

Cell adhesion and signalling during Drosophila myoblast fusion

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Myoblast fusion is a fundamental process during embryonic development to produce skeletal muscles. In the model organism Drosophila melanogaster two myoblast populations (founder myoblasts and fusion-competent myoblasts) are required for muscle formation. The identity of the muscle is determined by the fate of the founder myoblast, whereas the fusion-competent myoblasts provide the mass of the muscle. The recognition and adhesion of both types of myoblast requires members of the immunoglobulin superfamily (IgSF). They serve as a platform for the formation of a podosome-like signalling centre called FuRMAS. Upon myoblast recognition and adhesion signalling proteins transfer the fusion signal from the IgSF molecules to the actin cytoskeleton. As a result F-actin foci are visible at sites of cell contact.
Individual fusion events have been shown to occur within minutes. This requires the stabilization of cell adhesion as well as destabilization. We performed genetic and molecular screens to get further insights into the mechanism of myoblast adhesion and fusion. We identified a member of the Cadherin family that is present in both myoblast types. Cadherins may stabilize the contact between founder and fusion-component myoblasts initiated by IgSF members. We further gained evidence that SH2-SH3 adaptor proteins link the intracellular domain of the cell adhesion molecules to signal to the actin cytoskeleton.

Molecular mechanism regulating the morphogenesis and migration of slow muscle fiber in zebrafish

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The trunk muscle of the zebrafish embryo is composed of two distinct types of fibers, fast-twitch and slow-twitch, that can be distinguished by morphological and physiological criteria. Fast muscle fibers are multinuclear and constitute a majority of trunk muscle, whereas mononuclear slow muscle fibers are localized superficially. These muscle fibers originate from discrete progenitor pools that show distinct behaviours. The slow fibers derive from adaxial cells that are initially localized adjacent to the notochord but migrate through the myotome during somitogenesis. Fast fibers, by contrast, derive from more laterally located progenitors and are formed by cell fusion. It is well established that specification of slow muscle progenitors depends on Hedgehog signaling from the notochord but the molecular mechanisms regulating their migration and differentiation are still unclear. A key target of Hedgehog signaling in adaxial cells is the ubo gene, which encodes the transcription factor Prdm1a. Loss of ubo function not only disrupts slow twitch fiber differentiation but also affects the migratory and fusion behaviour of adaxial cells. I am investigating the link between Prdm1 function and these processes by manipulating candidate Prdm1a-target gene expression in transgenic embryos that allow real time imaging of the migration and fusion processes.

Identification of a long-term self-renewable population in activated satellite cells

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Muscle satellite cells are mitotically quiescent in muscle but are activated immediately following muscle injury, in order to proliferate for supplying myonuclei and self-renew for maintaining satellite cell pool. Although fast-dividing and slow-dividing cells in activated satellite cell population have been observed, the functional differences between them are unclear. To understand the relation between the proliferation behavior and stem cell function, we isolated satellite cells from mice and labelled with a fluorescent lipophilic dye PKH26, to chase the frequency of cell division. Time-lapse analysis revealed that a small population retaining high level of PKH26 dye exists even in the highly proliferative culture-condition. Both of fast-dividing PKH26low and slow-dividing PKH26high populations sorted by FACS underwent myogenic differentiation and self-renewal, whereas only PKH26high population was able to give rise to proliferative progeny having long-term self-renewal ability when passaged. Id1 protein, a negative regulator for bHLH transcriptional factors, was expressed in all undifferentiated satellite cells after activation but the expression level was varied even within PKH26high population. We found that the long-term self-renewable population is restricted to a compartment that expresses high level of Id1 protein (PKH26high/Id1high population). Finally, gene expression analysis identified the molecular characteristics of PKH26high/Id1high population. Taken together, our results suggest that the slow-dividing population expressing high level of Id1 protein in activated satellite cells retains long-term self-renewal ability, and the PKH26high/Id1high population may have indispensable roles for efficient repeated regeneration of adult muscle.

Segregation of myoblast fusion and muscle specific gene expression by distinct ligand-dependent inactivation of GSK-3β

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Myogenic differentiation involves myoblast fusion and induction of muscle-specific gene expression, which are both stimulated by pharmacological (LiCl), genetic or IGF-I-mediated GSK-3β inactivation. To assess whether stimulation of myogenic differentiation is common to ligand-mediated GSK-3β inactivation, myoblast fusion and muscle-specific gene expression were investigated in response to Wnt-3a. Moreover, crosstalk between IGF-I/GSK-3β/NFATc3 and Wnt/GSK-3β/β-catenin signalling was assessed. While both Wnt-3a and LiCl promoted myoblast fusion, muscle-specific gene expression was increased by LiCl, but not by Wnt-3a or β-catenin over-expression. Furthermore, LiCl and IGF-I but not Wnt-3a increased NFATc3 transcriptional activity. In contrast, β-catenin-dependent transcriptional activity was increased by Wnt-3a and LiCl but not IGF-I. These results for the first time reveal a segregated regulation of myoblast fusion and muscle-specific gene expression following stimulation of myogenic differentiation in response to distinct ligand-specific signalling routes of GSK-3β inactivation.

Structural determinants of Calpain-3 homodimerisation

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Calpains (CAPNs) are calcium-dependent cysteine proteases involved in the control of various cellular processes. They are generally known as regulatory proteases that modulate the biological function of their targets by acting in a selective and limited manner. Calpain-3 (CAPN3) is a tissue-specific member of the Calpain family which is predominantly expressed in skeletal muscles. It has been genetically linked to the limb-girdle muscular dystrophy type 2A (LGMD2A), a progressive muscle disease characterised by weakness and wasting in the hip, thigh and shoulder muscles. Despite a domain organisation similar to that of CAPN-1 and -2, the ubiquitous and best characterised members of the family, the CAPN-3 differs from these latter in several ways. We are currently focusing our efforts on the specific property of CAPN-3 to form a stable homodimer and its biological significance under both physiological and pathological conditions.

MKP-1 is essential for temporally controlled macrophage activation in tissue repair

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Tissue repair after injury requires the concerted action of inflammatory and tissue-specific cells in order to restore homeostasis. At early phases of muscle regeneration, infiltrating monocytes/macrophages display a pro-inflammatory gene expression program which at later stages changes to anti-inflammatory, promoting different responses on muscle stem cells. Here, we show a novel role for mitogen-activated protein kinase (MAPK)-phosphatase-1 (MKP-1) in tissue repair, via a paracrine action of macrophages over muscle stem cells: i) by restricting the outcome of the inflammatory response induced by MAPK activity; ii) by controlling the timely activation of the macrophage anti-inflammatory gene program, thereby promoting stem cell proliferation and subsequent growth of the newly formed myofiber; and iii) by preventing return of macrophages to resting state while tissue damage persists through interplay with the PI3K/AKT pathway.

A quantitative method to detect muscle functional recovery for both active and passive behavior

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Damaged muscle is known to lead to an organized process where satellite cells become activated, giving rise more satellite cells to finally restore injured fibers. Histological improvement has been considered for a long time as the recovery of the active behavior of muscle. Here, we have employed experimental methods to measure both in vivo contractile properties and in vitro passive stress-stretch behavior after snake venom-induced myotoxicity in rat tibialis anterior (TA) muscle and compare whether or not muscular function recovery means histological improvement. Two days after venom injection, the capacity of muscle to contract is disturbed in parallel to a degeneration process of muscle. Both myosin and actin decrease in injured muscles as muscular function is lost. Muscle respond leads to the activation of regeneration process, giving rise an increase of regenerating myofibers; however, contractile proteins are not recovered yet and muscular function is still compromised. Only when nuclei of new small myofibers return to the periphery of each fiber, losing the embrionary expression of myosin together with the gain of adult myosin and actin proteins, muscle is capable now to make force. Interestingly, although collagen accumulation is increased in damaged muscles, passive behavior is not affected. Counts of collagen I and III indicated that relation of these proteins is similar to controls, suggesting that composition of collagen rather than its accumulation may be the responsible of passive behavior. A better understanding of the mechanisms of muscle degeneration-regeneration may promote in the future appropriate treatment strategies in human muscular diseases.

Amniotic fluid stem cells restore the satellite cell niche when systemically injected in a model of spinal muscular atrophy

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Mutations in the survival of motor neuron gene (SMN1) are responsible for spinal muscular atrophy (SMA), a fatal neuromuscular disorder. Using a Cre-LoxP system, mice carrying a homozygous deletion of Smn exon 7 directed to skeletal muscle (HSA-Cre, SmnF7/F7 mice) present the main clinical features of human progressive muscular dystrophies. We have investigated whether amniotic fluid stem (AFS) cells are able to functionally restore muscle tissue.
Freshly isolated and expanded mouse AFS cells GFP+ were injected via tail vein in 3 months old HSA-Cre, SmnF7/F7 mice. Long term morphological analyses and secondary transplantation were performed to demonstrate the restoration of satellite cell niche.
Muscle tissue of surviving AFS cells treated animals was analyzed 15 months after cell injection. Morphological analyses demonstrated that treated muscle tissue was compact, with few central nucleated fibres (<1%). Moreover, 58% of myofibres were GFP+ in AFS cells treated mice. PCR analyses of GFP on different muscles (gastrocnemius, tibialis anterior and diaphragm) confirmed that the AFS cells had spread throughout the body engrafting in various skeletal muscle tissues. Finally, freshly isolated satellite cells from muscles of AFS cells transplanted primary hosts were injected into muscles of HSA-Cre, SmnF7/F7 secondary hosts. One month after secondary transplantation, all the mice in the AFS cell group had GFP+ fibres (31%).
This is the first study demonstrating the functional integration of non-muscle and non-embryonic stem-derived cells into the satellite cell niche. The AFS cells' ability to replenish the skeletal muscle stem cell niche could make them a promising treatment option for muscular dystrophy.

The role of intranuclear lamin complexes in Emery-Dreifuss Muscular Dystrophy

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The nuclear lamina, a scaffold structure at the nuclear envelope, gained attention when mutations in A-type lamins and in lamin-associated proteins were linked to a group of diseases termed laminopathies. We have previously shown that a nucleoplasmic complex of A-type lamins and LAP2alpha is involved in the regulation of satellite cells. Loss of LAP2alpha and subsequent disruption of intranuclear lamin complexes caused an increase in skeletal muscle progenitor cells in vivo, and upregulated stem cell-type gene expression and delayed differentiation of muscle satellite cells.

Here we investigate a mutant version of LAP2alpha (P426L) which was recently shown to be associated with EDMD. Despite normal localization of LAP2alpha and lamin A/C, primary patient fibroblasts containing P426L LAP2alpha protein show growth retardation and delayed cell cycle reentry after serum starvation. The effect on cell cycle progression and differentiation in myoblasts is currently tested.
Furthermore, in order to investigate the effect of LAP2alpha loss in an EDMD disease background, LAP2alpha knockout mice were crossed with a knock-in mouse model expressing the EDMD delK32 mutant form of lamin A/C. Mice homozygous for this mutation in Lmna die before weaning. Loss of LAP2alpha slightly extended life span. However, most of the phenotypes reported in LAP2alpha deficient and in delK32 mutant mice were unchanged in the double mutant mice. Overall our data suggest that the disease phenotype in delK32 lamin mice is mainly caused by an overwhelming structural defect in the lamina, and that the activity of the nucleoplasmic A-type lamin / LAP2alpha complex in satellite cells is barely affected.

Evolutionary conserved role of Nfix during muscle development: the first parallelism between primary and second myogenesis in mammals and zebrafish (Danio rerio)

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The skeletal muscle tissue in amniotes is formed by different classes of myogenic precursors that act in different steps: embryonic, fetal myoblasts and satellite cells. Recently, genes that are differentially expressed in murine embryonic and fetal myoblasts have been identified; in particular, the transcription factor Nfix is present only in fetal myoblasts while it is absent in embryonic myoblasts. Functional analyses of the Nfix gene in mouse evidenced that it is able both to activate the transcription of specific fetal genes (e.g. Mck, beta-enolase) and to inhibit specific embryonic genes (e.g. slow Myosin heavy Chain).
Zebrafish muscles share many features with mouse but embryos with external development have to grow faster than amniotes to escape from predators and find food. In fact, at just 24 hours post fertilization (24 hpf) a functional myotome has been formed with primary slow and fast fibers. Following this primary muscle wave, after 48 hpf, secondary slow fibers differentiate in several body locations. The primary and second myogenic waves in zebrafish have not been correlated with murine embryonic and fetal myogenic waves till now. By using a loss-of-function approach to specifically abrogate the gene function in vivo, we evidence for the first time the evolutionary conserved role of nfixa, the hortolog of Nfix in zebrafish, in slow muscle development. Interestingly, nfixa strongly regulates slow twitch fiber differentiation during the second myogenic wave, having no apparent role in the first myogenesis.

UNC-45 links myosin assembly to muscle proteostasis.

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Organization of the motor protein myosin into structures that perform muscle contraction is the result of a regulated multi step assembly pathway. A family of proteins containing a UCS domain is involved in myosin folding and plays a role at different stages of myosin thick filament assembly and maintenance. The founding member of the UCS family is the myosin-specific chaperone UNC-45, originally identified in Caenorhabditis elegans. Mutations in the unc-45 gene result in disorganized and reduced number of myosin-containing thick filaments causing a severe movement defect of adult worms (Epstein and Thomson, 1974). We have recently shown that the regulation of UNC-45 protein levels is critical for muscle development. The E3 enzymes CHN-1 and UFD-2 form a functional ligase complex that regulates UNC-45 turnover, important for proper muscle formation (Hoppe et al., 2004; Janiesch et al., 2007; Landsverk et al., 2007; Kim et al., 2008).
The molecular mechanism of myosin assembly into thick filaments by UNC-45 is not understood at present. Structure-function analysis combined with biochemical ubiquitylation assays should help to understand the role of UNC-45 in myosin assembly and how this is modulated by ubiquitylation.

JAM-packed with nuclei: zebrafish JAM-B and JAM-C are essential for myocyte fusion

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Skeletal muscle is composed of bundles of syncytial myofibres, each formed by the fusion of individual muscle precursors. Myocyte fusion has been well-characterised in Drosophila, thanks to extensive genetic screens and in vivo observation of the larval body wall musculature. Our understanding in vertebrates lags behind, however, and our current knowledge is largely limited to cell culture and orthology-based studies. Identifying cell surface receptors that are essential for fusion in vertebrates in vivo is an important step in elucidating the mechanism and regulation of this important developmental process.
The cell surface proteins JAM-B and JAM-C are a receptor:ligand pair best known for their roles in leukocyte extravasation, tight junction formation and cell polarity. Both proteins are expressed during embryogenesis, but their developmental function has not been previously described. Zebrafish embryos mutant for jamb or jamc show a severe block in myocyte fusion, resulting in an overabundance of mononuclear fast muscle fibres. Transplant experiments suggest that these proteins interact between myocytes to allow them to fuse. Moreover, slow muscle precursors in prdm1 mutants - which inappropriately fuse with other myocytes - ectopically express jamc but not jamb. These results demonstrate that jamb and jamc are a receptor:ligand pair critical for the initiation of fusion, and constitute the first vertebrate-specific interaction to be identified in vivo.

Analysis of fusion-deficient myoblasts in the mouse

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Skeletal muscle fibers are syncytia that arise by myoblast fusion. Myoblast fusion occurs during development and in the adult. It is required for growth and repair of muscle fibers, as well for the contractile capacity of the muscle. On a cellular level, fusion is characterized by adhesion, alignment of myoblast membranes, followed by the formation of a specialized membrane microdomain and an actin-rich scaffold associated with fusing membranes at the contact sites.
Genetic analyses performed in Drosophila revealed a molecular cascade that controls actin polymerization to be essential for myoblast fusion. This cascade comprises Rac GTPases, Rac regulators, WASp nucleation promoting factor, and the Arp2/3 complex. Recent genetic analyses done in our laboratory demonstrated that the function of Rac1 in myoblast fusion is conserved in the animal kingdom. Despite the identification of genes essential for myoblast fusion, the cellular events that occur during myoblast fusion are not fully understood and particularly the role of actin during myoblast fusion has remained unclear.
We aim to understand myoblast fusion on a mechanistic level. Based on our and other genetic data, we hypothesize that fusion is controlled by a molecular cascade that is initiated by integrin signaling. This signal results in the assembly of a Rac1/ Cdc42-based actin scaffold at sites of myoblast fusion. We will define the genetic epistasis of Rac1, Cdc42 and Itgb1 in order to test whether these molecules act in one pathway, and use state of the art imaging technology to follow the dynamics of Rac1/ Cdc42-based actin scaffolds and vesicle transport during fusion of control and mutant myoblasts.

The Role of Nfix in Post-Natal Myogenesis and Muscle Regeneration

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Nfix is part of a family of highly conserved proteins that function as transcriptional activators and/or repressors of cellular and viral genes. Members of this family are widely expressed in different organs, and Nfix is the most expressed isoform in muscle. Recently, it has been highlighted the central role of Nfix in driving the transcriptional switch between embryonic and fetal myogenesis. The aim of this work is to establish if and how Nfix plays a role in post-natal muscle development, focusing on physiopathological processes such as muscle homeostasis, growth, and regeneration. To approach this issue we have analyzed Nfix expression in Satellite Cells both in vivo and in vitro, characterized a Nfix null mouse model, and evaluated the regeneration capacity after injury of Nfix null mice, compared to their wt littermates. Results have shown that, in the absence of Nfix, muscle morphology is altered; moreover, it has been observed a general conversion to a slower-twitch phenotype. Finally, the regeneration capacity of Nfix null mice, compared to their wt littermates, is different, and characterized by a delay in regeneration. In addition to that, Nfix is expressed in all the regenerated fibers of the wt mouse, reinforcing the hypothesis that its presence is crucial for regeneration. All together, the data obtained demonstrate that Nfix is not only fundamental for the switch from embryonic to fetal myogenesis, but also a crucial regulator of the physiology of

the adult muscle. We are now working with two muscle-specific Nfix null models, in order to address the question of the specificity of the phenotype observed.

HMGB1 release and redox regulation during the remodeling of skeletal muscle

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HMGB1, a damage associated molecular pattern [DAMP] molecules, favors tissue regeneration via recruitment and activation of leukocytes and stem cells. Here we demonstrate, in a model of acute sterile muscle injury, that regeneration is accompanied by active ROS production counterbalanced and overcome by the generation of antioxidant moieties. Mitochondria are initially responsible for ROS formation. However they undergo rapid disruption with almost complete disappearance. Twenty-four hours following injury we observed a strong induction of MURF1 and Atrogin-1 ubiquitin ligases, key signals in activation of the proteasome system and induction of muscle atrophy. At later time points, ROS generation is maintained by non-mitochondrial sources. The antioxidant response occurs both in regenerating fibers and leukocytes that express high levels of free thiols and antioxidant enzymes, such as SOD-1 and thioredoxin. HMGB1, a protein thiol, weakly expressed in healthy muscles, increases during regeneration in parallel with the antioxidant response both in fibers and leukocytes. A reduced environment may be important to maintain HMGB1 bioactivity. Indeed, oxidation abrogates both muscle stem cell migration in response to HMGB1 and their ability to differentiate into myofibers in vitro. We propose that the early antioxidant response in regenerating muscle limits HMGB1 oxidation, thus allowing successful muscle regeneration.

Short Telomeres and Stem Cell Exhaustion Model Duchenne Muscular Dystrophy in mdx/mTR Mice

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Duchenne Muscular Dystrophy (DMD) is a devastating muscle degenerative disease which affects ~1/3500 of all males born. DMD is caused by mutations in dystrophin, a cytoskeletal protein essential for the membrane stability of myofibers in skeletal muscle. Absence of dystrophin results in increased fragility of the sarcolemma, leading to progressive loss of muscle function, paralysis and death in early life. The lack of animal models closely recapitulating the human disease has severely limited our understanding of its pathophysiology. Indeed, mice sharing the same genetic defect with DMD patients, mdx mice, only exhibit a mild phenotype and never reach the end-stage failure of skeletal muscle observed in humans. A major difference between mice and humans is telomere length (mice >40kb, humans ~10kb). We postulated that the mild dystrophic phenotype in mdx mice results from the ability of their muscle stem cells (MuSC) to meet the high regenerative demand of dystrophic tissues throughout their lifetime, due to their longer telomeres. To test this hypothesis, we crossed dystrophic mdx mice with mice lacking the RNA component of the telomerase enzyme Terc. This novel mouse model exhibits a severe muscular dystrophy phenotype, including profound muscle weakness, elevated serum creatine kinase levels, kyphosis, increased membrane permeability, progressive muscle atrophy and shortened lifespan. Their MuSC exhibit reduced proliferative and regenerative capacity both in vitro and in vivo and their progeny has shortened telomeres. These data suggest that DMD progression results from a cell autonomous failure of MuSC to regenerate dystrophic muscles due to critical telomere shortening.

Six transcription complexes in adult skeletal muscle fiber-type determination and maintenance

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Adult skeletal muscles are composed of slow- and fast-type myofibers, which possess different contraction speed properties, different metabolism and different sensitivity to muscle atrophy. Six homeoproteins are important transcription factors during myogenesis in mammals. However, the physiological role of Six1 in adult skeletal muscle is not yet established. We generated Six1loxP mice and mated them with transgenic mice expressing CRE recombinase under the control of the human skeletal actin (HSA) promoter. Six1 mRNA and protein were no more detectable in adult skeletal muscle of HSA-CRE-Six1lox/lox mice. To analyze their muscle fiber-type, myosin heavy chain subtypes expression was examined by immunohistochemistry and RT-PCR experiments. Myosin heavy chain slow (MYH7) mRNA expression was increased in soleus and gastrocnemius of HSA-CRE-Six1lox/lox mice, 100% of sol fibers expressing MYH7. Genome wide Affymetrix experiments performed on wild type and HSA-CRE-Six1lox/lox mice allowed us to identify target genes under the control of Six1 in sol and gas adult muscles. Among the genes whose expression is downregulated in both muscles, we identified Parvalbumin. ChIP experiments with adult skeletal muscle chromatin revealed that Six1 is bound upstream of Parvalbumin transcriptional start site in vivo. We identified two MEF3 DNA sequences in the 700bp promoter sequences of Parvalbumin, whose integrity is required for Six1 activation. Our data indicate that Six1 homeoproteins control myofiber phenotype and muscle contraction through the control of Parvalbumin and of MyHC genes expression, and more generally through the control of genes involved in Ca++ homeostasis in the adult myofiber.

History of Mrf4 expression reveals an unprecedented level of myogenic priming of the founders of adult muscle satellite cells

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Homeostatsis and regeneration of mammalian adult skeletal muscle is critically dependent on satellite cells. Juvenile satellite cells arise during late foetal stages in mouse, contribute to prenatal and postnatal muscle growth and enter mitotic quiescence in young adults. The origin of satellite cells in trunk and limb musculature has been traced to somitic paraxial mesoderm, while lineage-tracing studies revealed that head muscle satellite cells originate from cranial mesoderm. However, the precise identity of founder cells that generate adult muscle satellite cells is unknown. At the cellular level, (a) are satellite cells direct descendents of unique pool of founder cells in the somitic and cranial mesoderm, or (b) do they derive from diverse founder cells that participate in myogenesis throughout development. At the molecular level, a recent report showed that a small subset of satellite cells which have no antecedence of expression of Myf5, a critical muscle determination gene, have better self-renewal capacity compared to the majority that have expressed Myf5. Discordantly, another study reported that nearly all satellite cells expressed Myod. Thus, there is confusion regarding the regulatory cell state of satellite cell founders in terms of the level of commitment in the myogenic lineage. Here, we show using genetic labelling strategies that founders of satellite cells have a history of Mrf4 expression, a muscle determination and differentiation gene. This reflects an unanticipated level of myogenic priming of the ancestral pool of satellite cells. Our results on the timing of induction of Mrf4 in founders of satellite cells suggest that these cells do not arise directly from one specific developmental stem/progenitor population. Importantly, our findings uncouple the extent of lineage priming of founder cells and the functional heterogeneity in the adult muscle satellite cell pool and thus, have important implications to the understanding of regulatory state of tissue-specific stem cells.

Pericyte cell memory and myogenic commitment

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Pluripotent stem cells represent an open perspective for regenerative medicine. In principle, they feature unlimited proliferation and differentiation abilities. However, teratoma formation and low differentiation efficiencies still constitute important issues that must be fully investigated yet. Recently, induced pluripotent stem cells (iPSCs), generated from reprogrammed fibroblasts, have been demonstrated to have in vitro and in vivo myogenic potential when sorted for the SM/C-2.6 antigen. Furthermore, chimeric mice from mdx-iPSCs, carrying the dystrophin gene on an artificial chromosome, showed tissue-specific expression of dystrophin. Nevertheless, myogenic differentiation protocols and potential of iPSCs generated from different cell sources still present unanswered questions. We generated iPSCs from prospectively sorted MABs (MAB-iPSCs) are pluripotent as fibroblast-derived iPSCs (f-iPSCs). However, both teratoma formation and genetic cell manipulation assays identify a durable epigenetic memory in MAB-iPSCs, resulting in stronger myogenic commitment. Striated muscle tissue accounts for up to 70% of MAB-iPSC teratomas. Moreover, transfection with Pax3 and Pax7 induces a more robust myogenic differentiation in MAB-iPSCs than in f-iPSCs. Our data strongly suggest that iPSCs are influenced by

the cell type of origin and, when generated from myogenic stem cells, can increase the differentiation efficiency towards skeletal muscle, providing a pluripotent reservoir for muscular dystrophy treatment.

Fate mapping muscle fibers during axolotl limb regeneration

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During regeneration of the urodele amphibian, muscle dedifferentiation results in mononucleate cells in the blastema which contribute to the formation of new muscle fibers in the appendage. However, the functional contribution of muscle dedifferentiation versus muscle stem cell (satellite cells) activation and the molecular pathways that control these processes remain unclear. We have developed a method to exclusively and permanently label muscle cells and trace their long-term fate during axolotl limb regeneration.
To fate map cells derived from dedifferentiated muscle, we used Cre/LoxP mediated recombination in transgenic axolotls. The blastemas of inducible Cre animals were transplanted onto amputated limbs/blastemas of LoxP reporter (CAGGs-LoxP-eGFP-LoxP-Cherry) animals and the limbs were allowed to fully regenerate. During regeneration, myogenesis occurs via cell fusion in the blastema resulting in inducible Cre blastema cells fusing with LoxP reporter cells to form the regenerating muscle. Thus the muscle fibers in the regenerated limb contain the nuclei from both the LoxP reporter as well as the transplanted inducible Cre cells.
After full limb regeneration, animals were injected with Tamoxifen to induce Cre mediated recombination resulting in expression of the fluorescence protein Cherry in the limb muscles. Only muscle fibers expressed cherry after Cre induction and no Cherry fluorescence was observed in the satellite cells. The induced limbs were amputated through the Cherry positive muscle fibers and were allowed to regenerate again to determine quantitative contribution of cherry positive muscle in the regenerate.

Polycomb EZH2 Controls Self-Renewal and Safeguards Transcriptional Identity of Skeletal Muscle Stem Cells

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Polycomb group (PcG) proteins regulate differentiation of embryonic stem (ES) cells and maintenance of multipotent and progenitor stem cell populations. The PRC2 subunit EZH2 methylates histone H3 lysine 27 (H3K27me3) establishing an epigenetic mark that identifies repressed chromatin regions. Ablation of PRC2 members in ES cells impairs their differentiation and results in unscheduled expression of mixed cell lineage genes. While PcG establish and maintain positional patterning of the body axis through regulation of Hox genes in both Drosophila and mammals, their role in conferring cell identity by repressing inappropriate cell lineage-specific transcription in animal development has not been demonstrated. Indeed, derepression of mixed cell lineage genes does not occur in epidermal, neuronal, or pancreatic cells of Ezh2 conditional null mice.
We have generated mice in which Ezh2 was conditionally ablated in satellite cells (SCs) (Ezh2mKO). While EZH2 was dispensable for fetal muscle development, it was required for postnatal muscle growth and adult muscle regeneration by ensuring appropriate homeostasis of the SC pool. Unlike other progenitors cells, reduced H3K27me3 in Ezh2mKO SCs was accompanied by PolII recruitment and transcriptional activation of genes normally repressed in SCs and expressed in other cell lineages, including cardiac progenitors, retinal cones, neurons, and chondrocytes. Thus, our findings indicate that EZH2, by regulating proliferation and maintaining transcriptional identity of adult muscle stem cells, is an important molecular component of adult skeletal myogenesis.

Plasminogen activator inhibitor-1 is an essential regulator of muscular dystrophy-associated fibrosis

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Increasing muscle fibrosis constitutes the principal cause of death in Duchenne Muscular Dystrophy (DMD) patients, through mechanisms that are poorly understood. Plasminogen activator inhibitor-1 (PAI-1) is expressed at high levels in muscles of both DMD patients and mdx mice, a model of DMD, decreasing with age. Unexpectedly, genetic loss of PAI-1 in mdx mice anticipates fibrosis by promoting transforming growth factor-β (TGFβ) pro-fibrogenic functions while negatively affecting regeneration. Notably, in vivo pharmacological neutralization of uPA in PAI-1-deficient mdx mice blunts muscle TGFβ-dependent profibrogenic pathways, inhibits fibrosis and attenuates dystrophy progression. These fibrogenic mechanisms appear also functional in DMD fibrotic muscle. Thus, PAI-1 is a new element of the fibrogenic regulatory network that restrains fibrosis development in muscular dystrophy.

Analysis of transcriptional networks instructing adult myogenesis in Drosophila

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Muscle development proceeds through an evolutionarily conserved series of events, including myoblast generation and migration, myotube formation and attachment to tendons, and myofiber maturation. Transitions between these events mature stem-cell-like "naïve" precursors to fusion-competent migratory intermediates to syncytial tubes that execute sarcomerogenesis and form contractile myofibers. Despite a basic understanding of myoblast specification, patterning and fusion in the Drosophila embryo, relatively little is known about the regulation of muscle fiber maturation or the specification of the fibrillar versus tubular muscle fate in Drosophila adult muscles. We performed deep sequencing comparing mRNA expression in adult indirect flight (fibrillar) and leg (tubular) muscles. In agreement with previous microarray studies, we find tubular and fibrillar muscle specific gene expression. Most notably, we find multiple muscle-specific splice isoforms of structural and regulatory genes, suggesting that functional and structural differences between muscle types are in part orchestrated through the regulation of splicing. To identify the transcriptional networks and signaling effectors regulating adult myogenic transitions, we are performing deep sequencing on muscle tissues isolated at key developmental timepoints using laser-capture microdissection. Comparison of leg to flight muscles should elucidate developmental differences between tubular and fibrillar muscle, and comparisons between different developmental timepoints should identify key regulators and effectors driving myogenic transitions.

Stem Cell-Mediated Transfer of a Human Artificial Chromosome Containing the Entire Dystrophin Locus Ameliorates Muscular Dystrophy

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Duchenne Muscular Dystrophy (DMD) affects skeletal muscle leading to progressive paralysis and premature death: there is no therapy, but novel strategies are entering clinical experimentation. For both gene and cell therapy, gene-replacement is complex due to the large size of dystrophin gene (2.4Mb) and cDNA (14Kb). In recent years, intra-arterial mesoangioblast (MAB: vessel-associated stem cells) transplantation caused amelioration of DMD models: this was due to their ability to cross the vessel-wall. Cells similar to MABs were isolated from human skeletal muscle and are currently under allogeneic clinical experimentation. Autologous transfer of genetically-corrected cells would be desirable (since it would not require immune-suppression), but because of the dystrophin size, alternative gene-correction strategies are being tested. At variance with conventional gene therapy vectors, human artificial chromosomes (HACs) remain episomal (avoiding insertional mutagenesis) and carry large genetic regions with regulatory elements. Despite these promises, no evidence of efficacy has ever been reported for HACs.
We report the amelioration of a model of DMD by a novel strategy, combining HAC-mediated gene-replacement with MAB transplantation. We genetically-corrected MABs derived from the dystrophic mdx mouse (mdxMABs) with a HAC vector containing the entire human dystrophin gene (DYS-HAC). DYS-HAC expressing mdxMAB clones robustly engrafted dystrophic skeletal muscle upon transplantation and gave rise to many dystrophin-positive fibers and satellite cells, leading to a significant morphological and functional amelioration that lasted for most of the mouse life-span. Thus, HAC-mediated

gene transfer shows efficacy in a pre-clinical model of a genetic disease, setting the basis for future translation of this strategy for DMD.

The role of Pglym78 in Drosophila myogenesis: Glycolytic pathway promotes muscle differentiation

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A large number of genes involved in myogenesis has been described, however some gaps still remain in understanding late aspects of muscle differentiation. To fill in these gaps we took advantage of the high conservation of myogenic pathways among species and the availability of gene expression patterns in Drosophila and zebrafish to identify functionally uncharacterized genes expressed in muscular compartments. We used RNAi knockdown approach to test functions of the 25 identified genes and found that four of them have a role in late steps of myogenesis in Drosophila.
We then performed an in depth analysis of Pglym78 encoding glycolytic enzyme phosphoglyceromutase and involved in a human metabolic myopathy. Glycolysis is a major metabolic process providing energy for muscle contraction but surprisingly Drosophila Pglym78 displays muscle specific expression long before muscles start to contract and when attenuated leads to dramatic phenotypes suggesting muscle differentiation defects. Similar to Pglym78 virtually all glycolytic genes are expressed in developing muscles, and as show our data, play a role in proper muscle differentiation. It has been recently reported that glycolytic genes can negatively influence Notch pathway (Saj et al., 2010), known to repress muscle differentiation. We therefore tested effect of Notch activation in developing muscles and found that gain of Notch function mimics loss of Pglym78. Thus our data provide evidence that glycolytic pathway promotes muscle differentiation by repressing Notch revealing a link between metabolism and development.

Annotating New Genes: From In Silico Screening to Validations by Experiments

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In recent years, the advent of high-throughput analytical techniques, such as microarrays and serial analysis of gene expression (SAGE), has led to a rapid accumulation of biological data. The large size of databases precludes manual analysis and renders unsystematic approaches obsolete. To cope with these new challenges and to facilitate efficient data analyses, numerous academic and commercial software packages and databases have been developed. Yet, genes to which no biological function has been assigned compromise the usability of these data. To facilitate functionally annotating these so-called 'novel genes', an in silico screening of such genes has been developed by focusing especially on their expression patterns, namely their "tissue-enrichment" (Uchida et al. BMC Genomics 2009) and a knowledge database called "C-It" (http://C-It.mpi-bn.mpg.de) (Gellert et al. Bioinformatics 2010) has been developed. Emphasis has also been placed on "tissue-specific" isoforms by developing a tool to analyze Affymetrix's Exon Array, called "Exon Array Analyzer (EAA)" (http://EAA.mpi-bn.mpg.de/) (Gellert et al. Bioinformatics 2009).
With these algorithms and tools, ~1,000 genes are currently being annotated, which are enriched in a tissue but have not been characterized. To this end, a project called "1,000 Genes Project" has been initiated to functionally annotate these evolutionary-conserved, tissue-enriched genes with unknown functions using various model organisms (mouse, chicken and zebrafish) and in vitro models (ES cells). In order to benefit from this project, we are also screening for such genes with possible relations to human diseases by incorporating SNPs information. In this conference, we would like to share some of our preliminary data on evolutionary-conserved, tissue-enriched genes with unknown function.

Murine satellite cells after cloning are heterogeneous and possess distinct differentiation potential both in vitro and in vivo

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Satellite cell (SC) developmental plasticity has been the subject of intensive investigation and several studies suggest that SCs compartment may be heterogeneous. Recently, we identified and clonally characterize two main subpopulations of SCs in rat skeletal muscles which were distinct by their growth rate in low proliferative clones (LPC) and high proliferative clones

(HPC). In this study we uphold the presence of LPC and HPC also in mouse skeletal muscles and investigated whether they could have a different role for muscle regeneration.
Clones of SCs were obtained from mouse EDL and Soleus myofibers. After enzymatic digestion, muscle fibers were collected and triturated using a 18 G needle to disengage SCs. The resulting suspension was cloned through limiting dilution. MTS proliferative assay was performed at 5, 10 and 15 days of culture.
Proliferation rate, tested with MTS assay that highlighted a 6 and 9-fold increase in the dye processing by HPC compared to LPC respectively after 10 and 15 days in culture. Interestingly, when transplanted in cardiotoxin-injured Tibialis Anterior (TA) muscles, mouse GFP-positive LPC pools were able to reach a better engraftment than HPC, as confirmed by GFP detection through PCR analysis. Finally, only TA injected with LPC and not HPC were positive for GFP when transplantated with single clones expanded for 10 days.
These results support the suggestion that different proliferation and differentiation potential of HPC and LPC mirror two distinct SCs states in the myofibre. Their different in vivo regenerative ability may be relevant for cell therapy.

Role of collagen VI in skeletal muscle homeostasis.

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Collagen VI (ColVI) is an extracellular matrix protein, composed by three alpha-chains encoded by different genes. ColVI forms a microfilamentous network in the extracellular matrix of different tissues, including skeletal muscle where it localizes in the endomysium surrounding muscle fibers. Inherited mutations in ColVI genes cause in humans a number of muscle diseases, including Bethlem myopathy and Ullrich congenital muscular dystrophy. ColVI null mice (Col6a1−/−) display a myopathic phenotype with mitochondrial dysfunction, defective autophagy and spontaneous apoptosis of muscle fibers and other muscle cells. Analysis of muscle biopsies and primary cultures of UCMD patients revealed a similar phenotype, which could be normalized by treatment with cyclosporin A or its non-immunosopressive derivates.
Considering the remarkable apoptotic phenotype displayed by Col6a1−/− and UCMD myoblasts, we investigated muscle regeneration in Col6a1−/− mice. Histological analysis showed that under physiological conditions tibialis anterior muscles from Col6a1−/− mice display increased incidence of centrally nucleated myofibers and reduced myofiber mean cross sectional area (mCSA) than wild-type, with no obvious fibrotic changes. In order to investigate further muscle regeneration capability of Col6a1-/- mice, we analyzed the regenerative response after injury by cardiotoxin injection. After 7 days from injury, Col6a1-/- muscles showed delay in new myofiber formation when compared with wild-type muscles. After 30 days from cardiotoxin injection, muscles of both genotypes were able to re-establish tissue structure, but Col6a1-/- myofibers displayed increased mCSA when compared to wild-type muscles. We are currently investigating more in detail regeneration process and regenerated muscles of Col6a1-/- mice.

Gli2 and MEF2C activate each other's expression and function synergistically during myogenesis in vitro

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The transcription factors Gli2 (glioma associated factor 2), which is a transactivator of Sonic Hedgehog (Shh) signaling, and MEF2C (myocyte enhancer factor 2C) are concurrently expressed during myogenesis in vivo, and play important roles in the development of heart and skeletal muscle during embryogenesis. Both factors are able to activate cardiac and skeletal myogenic programs in vitro in a similar fashion. Although the physiological importance of Shh signaling and MEF2 family proteins in cardiac and skeletal myogenesis is known from previous studies in vivo, to date, the mechanistic understanding of their roles has not been clear. We demonstrate that Gli2 and MEF2C activated each other's expression while enhancing myogenesis in differentiating stem cells. Furthermore, dominant-negative chimeric proteins of either Gli2 or MEF2C repressed each other's expression, while inhibiting myogenesis in vitro. In addition, chromatin immunoprecipitation (ChIP) revealed direct binding of Gli2 to the Mef2c gene, and of MEF2C to Gli2 gene elements in differentiating P19 embryonal carcinoma (EC) cells. Finally, co-immunoprecipitation studies showed that Gli2 and MEF2C proteins formed a complex during myogenesis in P19 EC cells, capable of synergizing with MyoD on myogenesis-related gene promoters containing both Gli and MEF2 binding elements. We thus propose a model whereby Gli2 and MEF2C directly activate each other's expression and form a protein complex, which synergistically activates transcription, enhancing cardiac and skeletal muscle development. This model links Shh signalling to MEF2C function during cardiac and skeletal myogenesis.

High-intensity resistance training with insufficient recovery time changes the expression of muscle growth-related genes in rat

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Physical training promotes adjustments in the muscle fibers phenotype. However, high-intensity training associated with an inadequate recovery time may cause harmful effects that result in performance decrement. In this study we examined whether high-intensity resistance training with insufficient recovery time between bouts changes muscle fiber cross-sectional area (CSA), myogenic regulatory factors (MyoD and myogenin) and muscle growth factor (IGF1, insulin-like growth factor) gene expression in rat skeletal muscle. Wistar rats were divided into 4 groups: control 8- (C8), 12-weeks (C12) and trained 8- (T8), 12-weeks (T12), with T8 and T12 submitted to a high-intensity resistance training program (5 days/week), involving jump bouts into water, carrying progressive overloads based on percentage body weight. Analysis of plantaris muscle CSA using mATPase histochemical reaction, showed a significant reduction of the IIC and IIAD fibers types in T8 and IIA and IID in T12 group compared to C8 and C12, respectively. Gene expression of MyoD, myogenin and IGF1 determined by RT-qPCR showed the myogenin RNA gene expression similar among the groups. However, in T12 group, there was a decreased of MyoD and increased IGF1 gene expression, compared with C12 group. We conclude that high-intensity resistance training with insufficient recovery time, similar to overtraining conditions, induced plantaris muscle atrophy with decrease in MyoD mRNA levels, possibly indicating a lower activity of satellite cells during inadequate conditions of muscle repair. The increased IGF1 mRNA content may have occurred in an attempt to prevent the loss of muscle mass.
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Gene and protein expression of myosin heavy chain isoforms and meat tenderness in bovines longissimus dorsi muscle of different genetic groups

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Knowing that meat tenderness process which turns muscle into meat on postmortem are largely related with the myosin heavy chain (MHC) isoforms contentes, and in view of the needs to supply the livestock industry and consumer demands for a higher meat quality this study aimed to relate meat tenderness of Longissimus dorsi muscle (LD) with gene expression of MHC isoforms in two genetic groups of cattle. Steers of two genetic groups, Nellores (n=14) and Canchins (14) were used. The animals were maintained in the same physiological conditions (feeding, management and environment). After 134 days of feedlot, the animals were slaughtered and LD muscle samples were collected for meat tenderness analysis by the shear force and the myofibrillar fragmentation index analyzes and for MHC isoforms gene expression using RT-qPCR. The meat tenderness was better in Canchim in comparison to the Nellore groups; Canchim also showed a lower MHC 2X gene expression. There was a negative correlation between the gene expression of MHC 2A and 2X isoforms and a positive correlation between the gene expressions of MHC Slow isoform to meat tenderness in the genetic groups studied. Our results suggest that the lower meat tenderness in the LD of Nellore group could be related to the higher gene expression of MHC 2A and 2X in the Nellore group; the higher meat tenderness in the LD of Canchim group could be related to the higher gene expression of MHC Slow.

Novel helix-loop-helix transcription factor Mash4 maintains quiescent satellite cell population by suppression of MyoD activity

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Satellite cells are myogenic stem cells for postnatal muscle growth and muscle regeneration. MyoD, a myogenic basic-helix-loop-helix (bHLH) transcription factor, regulates satellite cell activation and differentiation. MyoD expression is not detected in quiescent satellite cells but is activated soon after muscle injury. To elucidate the molecular mechanisms of satellite cell self-renewal, we cloned a new type of bHLH factor, Mouse achaete-scute complex homolog-4 (Mash4) from satellite cells cDNA. RT-PCR and in situ hybridization data showed that Mash4 was specifically expressed in quiescent satellite cells. Ectopic expression of Mash4 suppressed formation of myogenin(+)/myosin heavy chain(+) differentiating myocytes and increased the number of reserve cells, a MyoD(-)/Pax7(+) quiescent satellite cell like population. Mash4 inhibited MyoD-dependent activation of E-box-driving luciferase reporter gene. In addition, Mash4 suppressed MyoD-upstream regulatory region-driving luciferase reporter genes, suggesting that Mash4 directly suppresses MyoD gene expression via inhibition of MyoD-auto-regulatory loop. Furthermore, Mash4 suppressed gene expression of Noggin, an antagonist of BMPs which play essential roles in satellite cell proliferation and self-renewal. Finally, Mash4-expressing myoblasts display significantly higher engraftment rate and an increased number of satellite cell contribution compared to the control myoblasts when injected into injured muscle. These results suggest that Mash4 is a novel transcription factor regulating the satellite cell self-renewal by inhibition of MyoD-dependent transcription along with suppression of MyoD gene expression and activation of BMP signaling.

Chronic TGFß antagonism improves respiratory function and skeletal muscle regenerative capacity in mdx mice

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TGF ß plays a key role in the pathophysiology of many fibrotic diseases including muscular dytrophies, and therapeutics that reduce the level of the cytokine may have clinical utility. Single dose administration of fresolimumab, an antibody neutralizing all three isoforms of TGFß to patients with primary focal segmental glomerulosclerosis in a phase 1 study demonstrated that the drug was well tolerated(1). We have investigated (2) the chronic treatment of mdx mice with 1D11 (a murine surrogate for fresolimumab) or with an antagonist to the angiotensin receptor (losartan). Significant increases in muscle fiber density were observed in mdx mice treated with 1D11, losartan, and a combination of the 2 drugs when compared to vehicle-treated controls. The 1D11-treated animals showed a slight increase in diaphragm muscle fiber density when compared with those treated with losartan. All treatment groups showed an increase in the total number of centrally nucleated fibers, suggesting an increase in fiber regeneration. Notably, respiratory function was normalized in all three treatment groups, however, forelimb grip strength was normalized only for 1D11-treated animals. Other indicators of improved function in treated animals include decreased serum creatine kinase and hydroxyproline levels – indicating improved sarcolemmal integrity and decreased fibrosis, respectively; increased number of myogenin-positive nuclei – suggesting more late-stage differentiated satellite cells. In conclusion, our studies indicate that TGF ß antagonism with 1D11 improves muscle regeneration and function in mdx mice.

1)Trachtman et al (2 March 2011) Kidney International.
2)Nelson et al (2011 in press) American Journal of Pathology

The Role of Numb in satellite cell mediated repair.

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Skeletal muscle repair is dependent on the balance between the self-renewal of myogenic satellite cells and their entry into the differentiation pathway that ultimately leads to a cessation of cell proliferation and expression of muscle structural genes. The molecular mechanism(s) regulating this binary switch is poorly understood. Numb is asymmetrically distributed during satellite cell division and co-localizes with the cell expressing muscle differentiation-specific genes. This predicts that Numb plays a critical role in regulating the binary decision between cell proliferation and differentiation. Using inducible null alleles of Numb and Numblike we determined that in the absence of Numb, cardiotoxin induced muscle injury resulted in dystrophic changes and increased fibrotic tissue. Satellite cells isolated from mice that harbor the inducible Numb null alleles demonstrate fewer, less robust myofibers when induced to differentiate in culture. In the absence of Numb, the satellite cells were demonstrated to have a proliferation defect that is not the result of increased apoptosis. Quantitative RT-PCR from Numb null and control satellite cells demonstrate altered gene expression. Transfection with gene specific siRNA restored proliferation. These data indicate that Numb may be important for initial activation and proliferation of satellite cells.

The role of LamininB2 during mesoderm differentiation and extracellular matrix formation in Drosophila

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As major component of basement membranes Laminin provides a scaffold for tissues and organs and plays an active role during a wide array of developmental and morphogenetic processes. In Drosophila, four Laminin genes had been identified that encode for two alpha chains (LamininA, LanA and Wingblister, Wb), one beta (LamininB1, LanB1) and one gamma subunit (LamininB2, LanB2), respectively. Here we report the identification of novel mutations in the Drosophila LanB2 gene, we originally discovered as second site mutation in a screen for genes that affect visceral muscle development. Since no mutations of LanB2 had been characterized so far, we describe their effects on embryogenesis, in particular the differentiation of mesodermal tissues with respect to the ECM. We show that the extracellular deposition of Laminin is blocked due to the loss of the LanB2 subunit resulting in an abnormal distribution of ECM components like Nidogen, Papilin, type IV Collagen and SPARC. The loss of LanB2 also impairs midgut formation thereby affecting endodermal cell migration, proper outgrowth of circular myotubes and guidance of longitudinal visceral muscles. Our data concerning the different functions of the two Laminin trimers during midgut development suggest independent roles for both trimers during ECM assembly although both trimers seem to act in a cumulative way during midgut development. Finally, the somatic muscle phenotype of LanB2 mutants is strongly enhanced in genetic interaction experiments with kon-tiki (kon) and thrombospondin (tsp) providing evidence for additional Laminin functions during somatic muscle target site recognition and the maintenance of muscle attachment.

ATOH8, a novel regulator of skeletal myogenesis and muscle regeneration

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Skeletal myogenesis and myogenic regeneration are essentially very similar processes that ensure that proper functional muscle tissue is formed during developmental growth and maintained in the course of postnatal life. Satellite cells being the main source of resident muscle stem cells are mainly responsible for the extensive muscle growth during late embryonic development and also for muscle regeneration in adult life. Studies on the activation and differentiation behavior of satellite cells has unveiled numerous parallels to embryonic myoblast, leading to an encouraging assumption that a better understanding of satellite cells would be highly instrumental in designing cellular therapies for muscular dystrophies. We show here for the first time that ATOH8, a bHLH transcription factor is expressed along with Pax7 in satellite cell as well as in skeletal myoblasts, cardiomyocytes and smooth muscle myoblast. Our results show that ATOH8 is expressed in activated satellite cells and is downregulated as cells enter terminal differentiation. Regenerating muscle shows an upregulated ATOH8 expression at site of injury. Furthermore, we show that ATOH8 is expressed in the myotome of chicken embryos and silencing of ATOH8 in chicken somites perturbs skeletal myogenesis. In all, our results show for the first time the involvement of ATOH8 in embryonic myogenesis and satellite cell differentiation. We conclude that ATOH8 is essential for the fine regulation of the essential balance between skeletal myogenesis and self renewal of satellite cell.

Identification of epigenetic modifiers involved in muscle stem cell differentiation

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Skeletal muscle stem cells or satellite cells within the myofibers are usually kept in a dormant state but can be activated by physical exercise and injuries of the muscle. Activation and subsequent proliferation of satellite cells response to physiological and pathological needs and maintain muscle homeostasis. Upon extrinsic activation, muscle stem cells dramatically change their chromatin organization, which makes muscle stem cells an excellent paradigm to study the epigenetic mechanisms underlying the transition from stem to differentiated cells. To unravel epigenetic markers that are capable of distinguishing muscle stem cells and differentiated myocytes we performed a histone modification antibody-based screening on isolated myofibers and identified two histone modifications H4K20me3 and H4R3me2s, which show complementary enrichment in nuclei of differentiated myocytes (H4K20me3) and satellite cells (H4R3me2s). Further molecular and cellular biological analysis in satellite cell derived C2C12 cells revealed that Suv4-20h2 and PRMT5, two enzymes that are responsible for these modifications, counteract each other in regulating muscle cell differentiation. Our results showed that PRMT5 and Suv4-20h2 mediated H4R3me2s and H4K20me3 respectively play a critical role in indexing chromatin organization during muscle stem cell differentiation, implying that a potential crosstalk between these two histone modifications is instrumental for regulation of muscle stem cells homeostasis. Future work will focus on the molecular mechanism that links histone H4 methylations to muscle stem cell differentiation, and the function of PRMT5 and Suv4-20h2 for developmental myogenesis and muscle regeneration in vivo.

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