

**PW 30:**  
**Myogenesis and cell  
transplantation**

PW30-372	<p><b>NUCLEAR MOVEMENT DURING MYOFIBER FORMATION</b>  CADOT B<sup>1</sup>, GACHE V<sup>1</sup>, GOMES E<sup>1</sup>  (1) INSERM U787, PARIS, FRANCE.</p>
To contact the author:: cadotbru@hotmail.com.	<p>Nuclear movement during myofiber formation</p> <p>The formation of a myofiber requires the fusion of myoblasts to form a myotube which then differentiate into a mature myofiber. During all these steps, the position of the nuclei changes: during the formation of the myotube, the nuclei from myoblasts move from the site of fusion to the center of the myotube. During myofiber formation, the nuclei move from the center to the periphery of the myotube. Moreover, in the mature myofiber, some nuclei become anchored close to the clusters of acetylcholine receptors, precursor of neuromuscular synapse. It has been proposed that the distribution of nuclei in the mature myofiber forms distinct functional domains and this is probably important for muscle function since mis-distribution of nuclei is observed during muscle regeneration and in some muscular disorders. The question of how the position of nucleus is established in muscle cells and what are the molecular mechanisms responsible for the position of the nucleus in muscle cells has not been addressed. To answer this question we are identifying the nuclear position events that occur during myotube formation, using both primary and immortalized myoblasts. We are using high content time-lapse microscope to determined the trajectories of nuclei after cell fusion. Using this approach, we will identify the cytoskeleton elements involved in nuclear position (actin and microtubules). Furthermore, we will characterize the pathways that regulate these nuclear movement events.</p>

PW30-373	<p><b><u>BARX2 REGULATES SATELLITE CELLS ACTIVATION AND DIFFERENTIATION DURING MUSCLE DEVELOPMENT AND REGENERATION.</u></b>  MEECH R<sup>2</sup>, PICKLE L<sup>1</sup>, GONZALEZ K<sup>2</sup>, MAKARENKOVA H<sup>1</sup>  (1) The Neurosciences Institute, San Diego, USA. (2) The Scripps Research Institute, La Jolla, USA.</p>
To contact the author:: makarenkova@nsi.edu.	<p>Satellite cells are muscle progenitor cells that are involved in normal muscle growth and provide a reserve capacity to replace damaged muscle fibers following injury or disease. However, the factors that regulate satellite cell self-renewal, activation and differentiation are not fully defined. In this study we show that homeodomain transcription factor Barx2 is a novel regulator of muscle development and repair. Barx2 is expressed in embryonic myoblasts and adult satellite cells and interacts with other muscle-expressed transcription factors. Barx2 is strongly upregulated in Pax7-expressing satellite cells after muscle injury suggesting a role for Barx2 in satellite cell activation. Consistent with this notion, mice lacking the Barx2 gene show reduced body and muscle mass and defective repair after acute muscle injury, as well as decreased Pax7 expression. In addition, loss of Barx2 in dystrophic <i>mdx</i> mice (Barx2/<i>mdx</i> double null) leads to a much more severe muscle phenotype than either parental strain alone. In satellite cell cultures, Barx2 regulates early events of differentiation and directly controls the expression of muscle-specific genes in cooperation with MyoD and SRF. Moreover, satellite cell cultures prepared from Barx2<sup>-/-</sup> muscle show a decreased proliferation rate and delayed differentiation together with downregulation of smooth muscle actin and other differentiation markers such as myogenin and myosin heavy chain, suggesting that Barx2 could control differentiation of satellite cells. Moreover, cultured Barx2<sup>-/-</sup> satellite cells show decreased substrate attachment and migration abilities. Taken together these data suggest that Barx2 is an important factor for satellite cell activation and differentiation.</p>

PW30-374	<p><b><u>REGULATION OF RESERVE CELLS BY ANGIOPOIETIN 1/TIE-2 SYSTEM WITHIN HUMAN MYOGENIC PRECURSOR CELL POPULATION</u></b>          ABOU-KHALIL R<sup>1</sup>, GHERARDI K<sup>1</sup>, CHAZAUD B<sup>1</sup>          (1) INSERM U841, Creteil, FRANCE.</p>
<p>To contact the author::          benedicte.chazaud@ins          erm.fr.</p>	<p>Satellite cells are now considered as true stem cells as they both participate to skeletal muscle repair by contributing to the formation of new myofibres and provide new quiescent satellite cells or reserve cells presenting the same properties. We have previously shown that in adult normal skeletal muscle, satellite cells are located close to capillaries. In vitro, endothelial cells and myogenic precursor cells (mpcs) have privileged interactions and may act in a paracrine way (Christov et al. 2007). One of the main molecular systems that regulate vascular homeostasis is the angiotensin (Ang)/ Tie system. Particularly, Angiotensin 1 (Ang1) binding to its tyrosine kinase Tie-2 receptor is required to maintain stabilization of the vessel and endothelial cell survival. Beside its role in the vascular system, Ang1/Tie-2 interaction has been also involved in hematopoietic stem cell quiescence by promoting their survival and maintain in the G0 phase.</p> <p>We have explored the role of Angs and Tie-2 system during in vitro myogenesis and in the regulation of myogenic cell population homeostasis. Expression of Ang1 decreased as cells differentiated, whereas Tie-2 expression increased. Both were upregulated in the reserve cells. Adding Ang1 to mpc cultures led to inhibition of both mpc growth and differentiation. Ang1 also protected mpcs from apoptosis through ERK1/2 signalling. These effects were mediated through Tie-2 receptor since a specific antagonist abolished Ang1 effect on mpcs. In whole mpc cultures, Ang1 treatment increased Pax7 expression and decreased MyoD expression. Inversely, silencing Tie-2 decreased Pax7 expression and increased MyoD expression, and increased the number of both proliferating and differentiating cells. In vivo, isolated quiescent satellite cells from both human and murine muscle expressed Tie-2 and Ang1. These results suggest that Ang1 binding to its receptor Tie-2 is involved in the regulation of the reserve cell pool within human myogenic precursor cell population.</p>

PW30-375	<p><b><u>DUAL ROLE OF PRO-INFLAMMATORY AND ANTI-INFLAMMATORY MACROPHAGES DURING SKELETAL MUSCLE REGENERATION AND MYOGENESIS</u></b></p> <p>YACOUB-YOUSSEF H<sup>1</sup>, ARNOLD L<sup>1</sup>, GHERARDI K<sup>1</sup>, CHAZAUD B<sup>1</sup>  (1) INSERM U841, Creteil, FRANCE.</p>
<p>To contact the author::  benedicte.chazaud@ins  erm.fr.</p>	<p>Macrophages are important for skeletal muscle regeneration and may exert beneficial effects on myogenic cell growth through mitogenic and anti-apoptotic activities. However, macrophages are highly versatile and may exert various, and even opposite, functions depending on their activation state. We have characterized the phenotype of monocyte/macrophages, studied their role during skeletal muscle regeneration and analyzed their effect on in vitro myogenesis.</p> <p>After injury, skeletal muscle recruited CX3CR1<sup>lo</sup>/Gr-1<sup>+</sup> monocytes from blood that exhibited a non-dividing proinflammatory profile. Then, within muscle, these cells switched their phenotype to become proliferating anti-inflammatory CX3CR1<sup>hi</sup>/Gr-1<sup>-</sup> cells that further differentiated into F4/80<sup>hi</sup> macrophages. We have shown that in vitro, phagocytosis of necrotic muscle cells induced a switch of proinflammatory macrophages toward an anti-inflammatory phenotype releasing TGFbeta1 suggesting that phenotype transition may occur in vivo upon phagocytosis of muscle debris. Depletion of circulating monocytes in CD11b-DTR mouse at time of injury totally prevented muscle regeneration, myofibres remaining in a necrotic state, indicating that macrophages are necessary for muscle repair. Depletion of the intramuscular F4/80<sup>hi</sup> macrophages at later stages after injury reduced the diameter of regenerating fibres suggesting that they are involved in myofibre growth.</p> <p>In vitro, we showed that pro-inflammatory macrophages stimulated human myogenic precursor cell (mpc) proliferation and prevented their differentiation whereas anti-inflammatory macrophages exhibited differentiating activity, assessed by myogenin expression and fusion into myotubes. We are investigating each step of in vitro myogenesis: migration, differentiation (expression of the myogenic programme) and fusion itself. Our first data showed that pro-inflammatory macrophages stimulated mpc migration and inhibited their differentiation and fusion. Inversely, anti-inflammatory macrophages slowed down mpc migration and stimulated their fusion into multinucleated myotubes.</p> <p>Finally, skeletal muscle regeneration would be sequentially associated with two main types of macrophages: first, inflammatory macrophages that sustain mpc proliferation, then anti-inflammatory macrophages that stimulate myogenesis and fiber growth.</p>

PW30-376	<p><b><u>STIM1 AND ORAI CHANNELS CONTROL EARLY POST-NATAL HUMAN MYOBLAST DIFFERENTIATION</u></b>  DARBELLAY B<sup>1</sup>, ARNAUDEAU S<sup>2</sup>, KÖNIG S<sup>2</sup>, JOUSSET H<sup>3</sup>, BADER CR<sup>1</sup>,  DEMAUREX N<sup>3</sup>, BERNHEIM L<sup>2</sup>  (1) Dept of Clinical Neurosciences and Dermatology - Geneva University Hospital, Geneva, SWITZERLAND. (2) Dept of Basic Neurosciences - University of Geneva Medical Center, Geneva, SWITZERLAND. (3) Dept of Cell Physiology and Metabolism - University of Geneva Medical Center, Geneva, SWITZERLAND.</p>
<p>To contact the author::  Basile.Darbella@medecine.unige.ch.</p>	<p>Specific intracellular calcium signals are required to induce myoblast differentiation. To generate these calcium signals, myoblasts can rely on two major sources: releases from intracellular calcium stores and influxes from extracellular medium. We have shown previously in cultured human myoblasts that store operated calcium entry (SOCE) is a possible source of calcium for differentiation to proceed. In the present study, we show that STIM1 and the Orai family of calcium channels regulate resting cytosolic calcium, intracellular calcium store level and SOCE in myoblasts. The inhibition of SOCE using siRNA strategy directed against the proteins STIM1 or Orai 1 and 3 fully prevented the expression of the transcription factor MEF2, a specific marker of myoblast differentiation. Conversely, MEF2 production was slightly accelerated by increasing SOCE with STIM1 and Orai1 over-expression. Despite a marked up-regulation during late differentiation, STIM1 and Orai1 are crucial only during the very first steps of the differentiation process as differentiation and fusion were not impeded by a 48h delayed SOCE knockdown. Inhibition of the different players of SOCE allowed us to show a strong correlation between the amplitude of SOCE measured at the onset of myoblast differentiation and the MEF2 expression, confirming that myoblast differentiation critically depend on the overall store operated calcium entry.</p>

PW30-377	<p><b><u>INVESTIGATING THE FUNCTION OF TSHZ3 DURING SKELETAL MUSCLE REGENERATION IN MOUSE.</u></b>  <b>FARALLI H<sup>1</sup>, CAUBIT X<sup>1</sup>, CORE N<sup>1</sup>, FASANO L<sup>1</sup></b>  (1) Institut de Biologie du Developpement de Marseille Luminy, Marseille, FRANCE.</p>
To contact the author:: faralli@ibdml.univ-mrs.fr.	<p>Muscle growth and repair depend on Satellite Cells (SCs), myogenic stem cells located between the plasma membrane and the basal lamina of the myofiber. When muscles are damaged, SCs become activated, proliferate and differentiate to form multinucleated myofibers. Skeletal muscle differentiation is initiated by the transcription factor MyoD, which binds directly to the regulatory regions of genes expressed during skeletal muscle differentiation and initiates chromatin remodelling at specific promoter. The modulation of the MyoD activity affects the balance between proliferation and differentiation of the activated SCs. The identification of the different players, which are implicated in this step, appears important.</p> <p>We found that, following treatment with cardiotoxin the zinc finger protein Tshz3 was strongly expressed in the SCs of regenerating adult skeletal muscle. Our immunohistochemical analyses indicated that Tshz3 is expressed in quiescent and activated SCs. In primary myofibers culture, Tshz3 expression was gradually downregulated when SCs differentiate into mature myofibers. Transfection and forced expression of Tshz3 in C2C12 myoblast cells resulted in delay of myogenic differentiation. Tshz3 might have a potential role in activation, proliferation and/or controlling differentiation of myogenic cells. Moreover, a luciferase reporter assay with the 184bp promoter of the Myogenin shows that Tshz3 represses MyoD dependent activation on this element.</p> <p>These results suggest that Tshz3 plays important roles in the molecular mechanisms operating in activated SCs when there are poised between proliferation and differentiation, probably through the regulation of the MyoD-dependent activation.</p>

PW30-378	<p><b>THE ROLE OF NECDIN IN THE MUSCLE REGENERATION</b></p> <p>FRANÇOIS S.<sup>1</sup>, DEPONTI D.<sup>2</sup>, PESSINA P.<sup>1</sup>, AZZONI E.<sup>1</sup>, MAGGIONI M.<sup>1</sup>, CLEMENTI E.<sup>2</sup>, MENEVERI R.<sup>1</sup>, COSSU G.<sup>2</sup>, BRUNELLI S.<sup>1</sup></p> <p>(1) Università Milano Bicocca- Dipartimento di Medicina Sperimentale, Monza, ITALY.  (2) SCRI-Stem Cell Research Institute-Dibit San Raffaele, Milano, ITALY.</p>
To contact the author:: s.francois@campus.uni mib.it.	<p>Necdin is a transcriptional co-factor of the MAGE protein family and deletion of this gene in human is associated with Prader-Willi syndrome (PWS). Mice lacking Necdin display a variety of phenotypes mimicking some aspects of the PWS. Previous studies suggested that necdin might act as growth suppressor in neurons, facilitating cell cycle exit and differentiation and inhibiting apoptosis. However Necdin role is not limited to the CNS and it has been demonstrated in our group that Necdin is also expressed during skeletal myogenesis, in myogenic precursor cells and when overexpressed leads to hypertrophy in C2C12 myoblasts.</p> <p>We have produced transgenic mice overexpressing Necdin under the control of MLC1F skeletal muscle specific promoter and we have also obtained, from our collaborators, a line of Necdin loss of function mice. We show that mice carrying the transgene show an accelerated capacity to regenerate damaged muscle fibres, while KO mice show decreased regeneration ability, accompanied by an increased level of apoptosis. These data indicates that Necdin plays an important role in muscle regeneration. Satellite cells are the main player in adult muscle regeneration and the molecular mechanisms playing a role in this process are similar to those involved in muscle development. We would like to get insight into the role of Necdin in the molecular regulation of satellite cells activation and differentiation.</p> <p>Muscle regeneration is also a key process in the development of cell therapy strategies for muscle dystrophy. Our actual aims are to identify the molecular partners of Necdin in the satellite cells using a two hybrid screening and GST-pull down and study how these interactions modulate Necdin activity during satellite cell activation and differentiation. We also intend to compare and study the differentiation properties and expression profiles of satellite cells derived from the Necdin gain and loss of function mice.</p>

PW30-379	<p><b><u>INVOLVEMENT OF CALPAINS IN MYOBLAST ADHESION AND MIGRATION</u></b>  ROUMES H<sup>1</sup>, DAURY L<sup>1</sup>, COTTIN P<sup>1</sup>, BRUSTIS JJ<sup>1</sup>  (1) Université Bordeaux I, Unité Protéolyse, Croissance et Développement  Musculaire, INRA USC-2009, avenue des Facultés, 33405 Talence, FRANCE.</p>
To contact the author:: hroumes@neuf.fr.	<p>Ubiquitous calpains (<math>\mu</math>- and m-calpain) are proteases of which enzymatic and structural properties are well characterized. Their implication in the early stages of myogenesis and more particularly in the fusion and migration of myoblasts seems well established. In the aim of improving myoblasts transplantation therapy, our study focuses on the role of the different actors of the proteolytic neutral calcium dependant system during adhesion and migration of murin (C2C12) and human (LHCN-M2) myoblasts.</p> <p>The adhesion of both cell lines is similar and rapid. In presence of calpain inhibitor, this phenomenon is delayed and reduced by about 55%. The characteristics of the migration phenomenon (cell morphology, velocity and migration area) have been analysed using video-microscopy. The morphology of both migrating cell lines as well as their migration velocity are similar. During this migration process, the global proteolytic activity of calpains has been measured on living cells using a fluorescent substrate (t-Boc-LM-CMAC). The calpain activity is higher in C2C12 cells than in LHCN-M2 myoblasts. The addition of a specific inhibitor decreases dramatically the velocity of myoblasts migration as well as the migration area of myoblasts.</p> <p>The impact of this inhibition has been observed on the actin cytoskeleton known to be the motor of migration. The stress fibres are disorganised, the cells have a rounded morphology and failed to form membrane protrusion.</p> <p>In conclusion, calpain activity seems to play a pivotal role for migration process and dispersion of human myoblasts. At the molecular level, these results suggest the implication of calpains in the organisation of stress fibres probably by a limited proteolysis of proteins involved in the organisation of the actin cytoskeleton.</p>

PW30-380	<p><b>OVERCOMING IMMUNE REJECTION IN MODELS OF TRANSPLANTATION IN DYSTROPHIC MUSCLE</b>  GROSS DA<sup>1</sup>, VIGNAUD L<sup>1</sup>, DA ROCHA RODRIGUES S<sup>1</sup>, GJATA B<sup>1</sup>, CHARLES S<sup>1</sup>, GEORGER C<sup>1</sup>, SCHERMAN D<sup>1</sup>, ISRAELI D<sup>1</sup>  (1) Genethon CNRS FRE 3087, Evry, FRANCE.</p>
To contact the author:: israeli@genethon.fr.	<p>Transplantation of muscle precursor cells (MPC) into dystrophic muscle is a major therapeutic approach in muscular dystrophies. However the clinical benefit of this approach is seriously compromised by the low survival rate of the transplanted cells. One principal reason for the low survival of transplanted MPC is their attack by the host immune response. Immune response in an allogenic transplantation is evoked principally by donor to host incompatibility of the major histocompatibility complex. However, mismatch of minor histocompatibility (mH) contributes to transplant rejection too. At present, the only way to overcome rejection is the continuous administration of non-specific immunosuppressive drugs, a treatment not without serious risk, which do not lead to transplantation tolerance.</p> <p>CD4+CD25+ regulatory T cells (Treg) are a unique subpopulation of CD4 T cells required for the maintenance of peripheral tolerance and with the ability to suppress deleterious immune response in many approaches.</p> <p>Here we demonstrate that immune response to the male mH antigen HY is sufficient to compromised survival of transplanted MPC. Importantly, infusing the host mouse with Treg specific for the male antigen HY, we were able to inhibit both the anti-HY immune response and the rejection process in two models of transplantation in muscle. Moreover, this induced immune tolerance was spread over to other rejection antigens presented in the engrafted cells, opening the way for large choice of therapeutic applications.</p>

PW30-381	<p><b><u>INTRAMUSCULAR MIGRATION OF SUBCUTANEOUSLY IMPLANTED MYOBLASTS IN NONHUMAN PRIMATES.</u></b>  SKUK D<sup>1</sup>, PARADIS M<sup>1</sup>, GOULET M<sup>1</sup>, TREMBLAY JP<sup>1</sup>  (1) Human Genetics Unit, CHUL Research Center, Quebec, CANADA.</p>
<p>To contact the author::  Daniel.Skuk@anm.ulaval.ca.</p>	<p>The main constraint of the therapeutic strategy of intramuscular myogenic-cell delivery is that the transplanted cells fuse only with the myofibers reached by the injection trajectories. This phenomenon is traditionally attributed to a "lack of migration" of the implanted cells. However, recent observations of the author in monkeys suggest that intramuscularly injected myoblasts always fuse with myofibers following an intra-fascicular migration, although this migration seems to be only towards the myofibers damaged by the injections. To confirm this migration capacity of the implanted myoblasts, we perform an experiment in nonhuman primates, the animal model more appropriate for human extrapolations in the field of myoblast transplantation. Skeletal muscles of cynomolgus monkeys were damaged with a 27G needle (100 parallel penetrations per cm<sup>2</sup>) and 1 hour later 1/2 ml of a cell suspension of allogeneic beta-galactosidase-labeled myoblasts was injected subcutaneously over the damaged region and over non-damaged regions. Monkeys were immunosuppressed with tacrolimus. One month later, the damaged regions were biopsied and analyzed by histology. In the damaged regions, beta-galactosidase-positive myofibers were observed up to 1 cm distant from the muscle surface (the depth of each needle penetration). The distribution pattern of the beta-galactosidase-positive myofibers followed the pattern of the previous needle trajectories. This indicates that in nonhuman primates the implanted myoblasts are capable to migrate for long distances into the recipient's muscle, although to reach and to fuse with damaged myofibers. The problem is thus not a "lack of migration capacity" but rather the incapacity to diffuse throughout a non-damaged muscle to fuse indiscriminately and spontaneously with non-regenerating myofibers.</p>

PW30-382	<p><b><u>MONITORING CELL TRANSPLANTATION PROTOCOLS BY 1H-NMR IMAGING : WHICH CLASS OF CONTRAST AGENT?</u></b>  VAUCHEZ K<sup>1</sup>, BALIGAND C<sup>2</sup>, VILQUIN JT<sup>3</sup>, FISZMAN M<sup>3</sup>, CARLIER P<sup>2</sup>  (1) Genzyme SA, Saint Germain en Laye, FRANCE. (2) NMR Laboratory, Association Institute of Myology and CEA, IFR14, Pitié-Salpêtrière University Hospital, Paris, FRANCE. (3) Inserm, U582, Institut de Myologie, UPMC Univ Paris 06, UMR S582, IFR14, Paris, FRANCE.</p>
To contact the author:: jt.vilquin@institut-myologie.org.	<p><b><u>Purpose:</u></b> High spatial resolution and non-invasiveness feature NMR imaging, and may allow longitudinal assessment of cell therapies. However, cells must be pre-loaded with an appropriate NMR contrast agent (CA). We compared the ability of two classes of CA, iron oxide nanoparticles (SPIO) and lanthanide complexes (Gd-DTPA), to monitor the fate of labeled myoblasts transplanted in a xenogenic context.</p> <p><b><u>Methods:</u></b> Primary human myoblasts were loaded by direct incubation with 25mM of Gd-DTPA (Magnevist) or 100µg/mL of SPIO (Endorem). 2.10<sup>6</sup> labelled cells were injected in the <i>tibialis anterior</i> of C57/Bl6 immunocompetent mice. NMR acquisitions were performed in a 4T Bruker Biospec NMR spectrometer. Interleaved axial T1-weighted spin echo images (in-plane resolution:120x120µm) were acquired immediately after cell injection (D0) and repeated on D1, D4, D6, D8, D11, D14, D21 and 3 months after transplantation. Size and contrast of the labeled area were measured. Comparatively, muscle cryosections were labeled with anti-human COX-2 (mitochondrial localization) and lamin A/C (nuclear localization) antibodies (immunohistofluorescence study). Prussian blue staining detected SPIO in tissue.</p> <p><b><u>Results:</u></b> T1-weighted images showed hyper-intensity (Gd-DTPA) and hypo-intensity (SPIO) signals at injection site on D0. The size of SPIO spots halved between D1 and D11 and plateaued thereafter, while the contrast remained stable over time. The Gd-DTPA label significantly decreased and totally disappeared at D21. Immunolabelings performed on D8 and D11 confirmed the immunorejection of human cells as expected. However, Prussian blue staining revealed the presence of SPIO at D8, D11 and at 3 months, in agreement with the persistence of the NMR signal.</p> <p><b><u>Conclusions:</u></b> Time-courses of CAs detection were different after intramuscular injection of loaded cells and the remanence of Gd-DTPA exceeded the known survival of xenografted loaded cells. Most importantly, SPIO label was still visible after 3 months, confirming its poor relevance for therapeutic cell monitoring <i>in vivo</i>. Supported by grants from AFM.</p>

PW30-383	<p><b><u>GENE EXPRESSION PROFILING OF PURIFIED ADULT SKELETAL MUSCLE PROGENITOR CELLS</u></b>  PALLAFACCHINA G<sup>1</sup>, MONTARRAS D<sup>1</sup>, CUMANO A<sup>2</sup>, BUCKINGHAM M<sup>1</sup>  (1) Pasteur Institute, Dept. Dev. Biol., Paris, FRANCE. (2) Pasteur Institute, Dept. Immun., Paris, FRANCE.</p>
To contact the author:: giorgiap@pasteur.fr.	<p>Satellite cells are the main progenitor cells for skeletal muscle growth and regeneration under physiological conditions. The difficulty of purifying these quiescent cells in sufficient number has precluded their biochemical characterization. Pax3 and Pax7 paired-box transcription factors play critical roles in skeletal myogenesis by directing progenitor cells into the myogenic programme and by ensuring their survival. In adult muscle, satellite cells are marked by the expression of Pax7 and, in a subset of muscles, also of Pax3. The generation of a <i>Pax3</i><sup>GFP/+</sup> mouse line permitted the purification of satellite cells expressing the GFP marker by flow cytometry and the demonstration of their major role in muscle repair together with their capacity to self renew as adult muscle progenitor cells <i>in vivo</i> (Montarras et al, 2005). This purification procedure has opened the way for biochemical characterization of these freshly isolated <i>Pax3</i><sup>GFP/+</sup> quiescent cells. The gene expression profile of quiescent satellite cells has been established by comparison with activated cells. Activated cells were obtained in three ways: <i>in vitro</i>, by culturing freshly isolated cells for 3 days in growth medium, and <i>in vivo</i>, by isolating them from growing post-natal muscles and from <i>mdx</i> mice. These mice lack dystrophin, a structural protein that is mutated in Duchenne muscular dystrophy patients. In <i>mdx</i> mice, fibre degeneration is chronic but constantly compensated by regeneration, which results from the continuous mobilization and activation of satellite cells. This approach gives new insights into satellite cell function and regulation and should lead to the identification of novel specific markers such as those of use for isolating satellite cells from other species, including humans.</p> <p>Montarras et al., Science 2005;309(5743):2064-7.</p>

PW30-384	<p><b>A NEW ROLE FOR SRF IN SKELETAL MUSCLE AGING</b>  LAHOUTE C<sup>1</sup>, SOTIROPOULOS A<sup>1</sup>, FAVIER M<sup>1</sup>, GUILLET-DENIAU I<sup>1</sup>, SCHMITT A<sup>1</sup>, METZGER D<sup>2</sup>, TUIL D<sup>1</sup>, DAEGELEN D<sup>1</sup>  (1) Institut Cochin, INSERM U567 CNRS UMR8104, PARIS, FRANCE. (2) Institut de Génétique et de Biologie Moléculaire et Cellulaire (IGBMC) INSERM/CNRS/ULP, ILLKIRCH, FRANCE.</p>
<p>To contact the author::  lahoute@cochin.inserm.fr.</p>	<p>The Serum Response Factor (SRF) is a crucial transcription factor for muscle-specific gene expression. We previously demonstrated that SRF is needed for postnatal skeletal muscle growth.</p> <p>To further investigate the role of SRF in adult skeletal muscle maintenance and regeneration, we developed a new model of knock-out mice allowing tamoxifen-inducible muscle-specific loss of SRF. We could thus trigger a rapid and efficient loss of SRF expression in post-mitotic myofibers, leading to the rapid down regulation of transcripts coding for SRF known target genes such as skeletal and cardiac alpha-actin as well as muscle creatine kinase. We also confirm in this model the importance of SRF expression for IGF-1 gene transcription.</p> <p>Muscles lacking SRF displayed no obvious phenotype until 5 months after tamoxifen injection. However SRF-KO muscles later developed an atrophy due to the reduction of myofiber cross-section area, a sarcomere disorganization and an aggravated accumulation of tubular aggregates. Moreover, these muscles displayed an altered lipid metabolism showing inter- and intra-myofiber lipid accumulation, particularly in oxidative fibers. After cardiotoxin injury, SRF-KO muscles also exhibited an altered regeneration process, showing smaller regenerated fibers and a persistent fibrosis. This phenotype is strongly reminiscent of age-related skeletal muscle abnormalities, suggesting that triggering SRF loss could accelerate the aging process.</p> <p>Interestingly, in skeletal muscle of control mice, we could observe a progressive and important decrease in SRF protein expression associated with muscle aging.</p> <p>Our data suggest that a naturally occurring SRF down-regulation or an induced SRF loss could contribute to the muscle aging process. The molecular mechanisms underlying such an accelerated muscle aging in the absence of SRF are under investigation.</p>

PW30-385	<p><b><u>ENHANCED MUSCLE RECONSTRUCTION AND SELECTION OF STEM-LIKE CELLS BY MYOGENIC MACROPHAGE-SECRETED FACTORS</u></b>  MALERBA A<sup>1</sup>, VITIELLO L<sup>1</sup>, SEGAT D<sup>1</sup>, DAZZO E<sup>1</sup>, FRIGO M<sup>1</sup>, SCAMBI I<sup>1</sup>, DE COPPI P<sup>2</sup>, BOLDRIN L<sup>2</sup>, MARTELLI L<sup>1</sup>, PASUT A<sup>1</sup>, ROMUALDI C<sup>1</sup>, BARONI D<sup>1</sup>  (1) Department of Biology, University of Padova, Padua, ITALY. (2) Department of Pediatrics, University of Padova, Padua, ITALY.</p>
To contact the author:: baronimd@bio.unipd.it.	<p>Skeletal muscle regeneration relies onto a specific population of myogenic precursors, named satellite cells. Inflammation also has a determinant role, as upon injuring macrophages are attracted by the damaged myofibers and the activated satellite cells and act as key elements of dynamic muscle supportive stroma. Yet, it is not known how macrophages interact with the more profound stem cells of the satellite cell niche. In this study we show that in the presence of a murine macrophage conditioned medium (mMCM) a subpopulation of stem-like cells could be selected and expanded from adult rat muscle with serial platings. These cells were small, round, poorly adhesive, slow-growing and showed mesenchymal differentiation plasticity. mMCM also inhibited the mesenchymal potential towards adipogenesis of satellite cells mechanically isolated from suspensions of single myofibers. mMCM-treated myogenic cells in mixed primary muscle cultures from neonatal rats showed a growth rate increase, spindle-like morphology and alignment before forming an impressive array of contracting myotubes; comparison with cultures from adult muscles suggested that mMCM-sensitive cells are more abundant in developing muscles. In vivo, intramuscular administrations of concentrated mMCM in a model of extensive surgical ablation of rat tibialis anterior dramatically improved muscle regeneration. Altogether, these findings suggest that macrophagic factors could be of great help in developing therapeutic protocols with myogenic stem cells.</p>

