

**PW 29:  
Cell therapies –  
Muscle embryogenesis**

PW29-357	<p><b>THE COFACTOR VESTIGIAL-LIKE 2 IS ASSOCIATED WITH SKELETAL MUSCLE DIFFERENTIATION IN CHICK LIMBS</b>  BONNET A<sup>1</sup>, DAI F<sup>2</sup>, BRAND-SABERI B<sup>2</sup>, DUPREZ D<sup>1</sup>  (1) Université Pierre et Marie Curie (ParisVI), CNRS, UMR7622, Laboratoire de Biologie du Développement, Paris, FRANCE. (2) Institute of Anatomy and Cell Biology, Department of Molecular Embryology, University of Freiburg, Freiburg, GERMANY.</p>
To contact the author:: aline.bonnet@snv.jussieu.fr.	<p>Skeletal muscle formation is crucially dependent on four basic-helix-loop-helix (bHLH) transcription factors, Myf5, MyoD, Mrf4 and Myogenin, which are named the myogenic regulatory factors (MRFs). Beside this recognized master role of the MRFs in triggering myogenesis in Vertebrates, there is emerging evidence that other transcription factors are important for muscle formation. Mammalian Vestigial-like 2 (Vgl2), a cofactor of TEF-1 and MEF2 transcription factors, has been shown to promote skeletal muscle differentiation <i>in vitro</i>.</p> <p>We analyzed the expression of Vgl2 transcripts during muscle formation in chick limbs. Vgl2 is expressed specifically in embryonic skeletal muscles. Comparison of Vgl2 transcript location with that of the known muscle markers such as Pax3, FgfR4, MyoD at different stages of limb development showed that Vgl2 transcripts are not expressed in muscle precursors migrating from the dermomyotome to the limb. Vgl2 expression starts at E4, in a domain similar to that of MyoD. At this stage, Vgl2 is expressed in a subpopulation of MyoD positive cells as shown by double <i>in situ</i> hybridization. When differentiated fibers appear, Vgl2 transcripts are present both in myoblasts and muscle fibers as are MyoD transcripts. We have previously shown that activation of the Notch pathway inhibits muscle differentiation without affecting the expression of the Pax3 and Myf5 genes. Similarly to the expression of MyoD, that of Vgl2 is clearly down regulated after Notch activation. The transcriptional relationship between MyoD and Vgl2 was analyzed in the neural tube and somite contexts by using electroporation. Experiments in both contexts showed that Vgl2 is a target of MyoD.</p> <p>All these results indicate that Vgl2 is involved in muscle differentiation downstream of MyoD. We are currently performing gain and loss of function experiments in chick embryos in order to understand Vgl2 function in myogenesis.</p>

PW29-3 58	<p><b>FUNCTIONAL CHARACTERIZATION OF SMALP IN SKELETAL MUSCLE</b>  POMIÈS P<sup>1</sup>, BECKERLE M<sup>2</sup>  (1) CRBM - CNRS UMR5237, Montpellier, FRANCE. (2) Huntsman Cancer Institute - University of Utah, Salt Lake City, USA.</p>
<p>To contact the author::  pascal.pomies@crbm.cnrs.fr.</p>	<p>The two ALP (<math>\alpha</math>-actinin associated LIM protein) isoforms, smALP and skALP, which exhibit identical N-terminal PDZ domains and C-terminal LIM motifs with a variable central core, are produced as a result of alternative splicing and are known to interact with the actin-crosslinking protein, <math>\alpha</math>-actinin. skALP is specifically expressed in adult skeletal muscle while smALP was supposed to be only expressed in smooth and cardiac muscles. Recently, using an antisense RNA strategy, we have shown that disruption of ALP isoform expression severely affects muscle differentiation (Pomiès et al., 2007).</p> <p>Here, using a novel isoform-specific antibody directed against smALP, we show that smALP expression is strikingly upregulated upon induction of differentiation of C2C12 cells and that smALP is also expressed in adult skeletal muscle. Furthermore, using indirect immunofluorescence microscopy, we show that upregulation of smALP expression at the early stage of myogenesis is concomitant with its translocation from the cytoplasm to the nucleus of C2C12 cells. This nuclear localization is confirmed by cellular fractionation using differentiating C2C12 cell lysates.</p> <p>Actually, using transitory transfections of C2C12 cells, we are mapping the domain of smALP that target the protein to the nucleus of differentiating skeletal muscle cells. Different smALP sequences such as the PDZ domain, the LIM motif and the unique central region of smALP coupled to a 6-histidine tag are expressed in C2C12 cells and are visualized by immunofluorescence microscopy. Furthermore, knowing that <math>\alpha</math>-actinin is the only ALP-interacting protein described to date, we are actually searching for smALP binding partners. We are therefore using an immunoprecipitation strategy to identify the binding partner repertoire of smALP from differentiating C2C12 nuclear extracts. Our hypothesis is that smALP is a key regulator of skeletal muscle differentiation which is translocated into the nucleus of myoblasts upon induction of differentiation in order to participate to the transcription machinery.</p>

PW29-359	<p><b><u>KIR2.1-LINKED HYPERPOLARIZATION SPECIFICALLY CONTROLS MEF-2A AND MEF-2C EXPRESSION DURING HUMAN PRIMARY MYOBLAST DIFFERENTIATION</u></b>  <b>KONIG S<sup>1</sup>, BADER CR<sup>2</sup>, BERNHEIM L<sup>1</sup></b>  (1) Department of Basic Neurosciences, Geneva, SWITZERLAND. (2) Department of Clinical Neurosciences and Dermatology, Geneva, SWITZERLAND.</p>
To contact the author:: stephane.konig@medecine.unige.ch.	<p>Our work is based on human primary myoblast cultures derived from single satellite cells. Human myoblasts are able to proliferate for several weeks in culture, and terminal differentiation into myotubes can be induced by serum withdrawal. In human, as in other species, it is well known that myoblast differentiation is mainly controlled by two families of transcription factors, the myogenic bHLH family (including MyoD and myogenin) and the MEF-2 (A-D) family.</p> <p>We have previously shown that, during the differentiation process of human myoblasts, the expression of myogenic transcription factors follows a hyperpolarization of the membrane myoblast resting potential to -70 mV due to Kir2.1 channel activation. Inhibition of this hyperpolarization strongly decreases myogenic transcription factor expression and myoblast differentiation, indicating that the hyperpolarization is a prerequisite for myogenic factor expression. We have shown, in addition, that the Kir2.1-linked hyperpolarization initiates the differentiation process by generating a cytoplasmic calcium signal which specifically activates the calcineurin pathway, although p38-MAPK, PI3K and CaMK pathways are also required for an optimal myoblast differentiation. When induced to differentiate, human myoblasts first hyperpolarize and then express myogenin and, slightly later, MEF-2A and MEF-2C. MEF-2D is already present during proliferation, although its expression increases during differentiation.</p> <p>Recently, we observed that preventing the Kir2.1-linked hyperpolarization with 10 mM cesium inhibited MEF-2A and MEF-2C expression whereas myogenin and MEF-2D expression were unaffected. These results suggest that the Kir2.1-linked hyperpolarization and the related calcium signal activating the calcineurin pathway triggers MEF-2A and MEF-2C, while myogenin and MEF-2D must be controlled by other pathways. We thus propose that the initiation of the differentiation process via activation of the calcineurin pathway mainly occurs through the induction of the expression of two specific members of the MEF-2 transcription factors, MEF-2A and MEF-2C.</p>

PW29-360	<p><b>MOLECULAR MECHANISMS OF KIR2.1 ACTIVATION AT THE ONSET OF HUMAN MYOBLAST DIFFERENTIATION</b></p> <p>HINARD V<sup>1</sup>, LEROY M<sup>1</sup>, BADER CR<sup>1</sup>, BERNHEIM L<sup>1</sup></p> <p>(1) University of Geneva, Geneva, SWITZERLAND.</p>
<p>To contact the author:: marina.leroy@medecine.unige.ch.</p>	<p>Myoblasts are mononucleated cells that fuse together to form skeletal muscle fibers. We have shown previously that human myoblast differentiation requires a hyperpolarization of the resting membrane potential to take place. This hyperpolarization is due to the activity of particular potassium channels, the Kir2.1 channels. Kir2.1 activation is, so far, the earliest detectable event during human myoblast differentiation. The purpose of this work was to elucidate the molecular mechanisms controlling Kir2.1 activation at the onset of differentiation.</p> <p>Last published results showed that Kir2.1 activity, as well as myoblast fusion, can be modulated by inhibitors of tyrosine kinases and tyrosine phosphatases. We observed that Kir2.1 channels are phosphorylated on tyrosine 242 in proliferating myoblasts, and that this phosphorylation decreases significantly during the first hours of differentiation. We thus proposed that phosphorylation of the tyrosine 242 maintains Kir2.1 channels in an inactive state during the proliferating phase of human myoblasts.</p> <p>Other groups, in other cell types, have shown that the Kir2.1 channels can be inactivated through EGF receptor activation. We therefore looked, in human myoblasts, whether receptor tyrosine kinases (RTKs) could be involved in Kir2.1 phosphorylation. Our first results suggest that the activation state of EGF/ErbB receptors and, to a lesser extent, Insulin/IGF receptors vary between proliferation and differentiation. We also observed that application of a high dose of EGF or surexpression of EGF-R slow down the differentiation process. Based on these preliminary data, we plan 1) to analyze the effect of EGF-R modulation on the Kir2.1 current and 2) to determine how the EGF-R activation is modulated during differentiation (internalization, heterodimerization).</p>

PW29-361	<p><b><u>TISSUE AND MOLECULAR INTERACTIONS REGULATING THE ONSET OF MYOGENESIS</u></b>  MIMAUT B<sup>†</sup>, CHERAUD Y<sup>1</sup>, FONTAINE-PERUS J<sup>1</sup>  (1) UMR CNRS 6204 Biorégulation, NANTES, FRANCE.</p>
<p>To contact the author::  benoit.mimault@etu.univ-nantes.fr.</p>	<p>The tissular and molecular interactions that govern the onset of myogenesis during embryogenesis are not yet entirely elucidated. The aim of our research is to use the advantages of the chick embryo to analyze the emergence of myoblasts in early muscle development. We believe that the in vivo manipulation remains a powerful technique allowing to discriminate the role played by a tissue in an embryonic process. In this context, the chick embryo is a model of choice to perform the manipulations since it is readily accessible to experimentation. We wish to identify the neural signals that participate in myogenesis by gain and loss function approach using electroporation and mouse-chick chimera method. Our experiments are specifically addressed to the transcription factor Pax3. Caudal neural tube of 1,5 day-old chick embryos are electroporated with constructs inducing over and under expression of the Pax3 gene. In our mouse-chick chimeras, reciprocal exchanges of precisely defined regions of neural tubes are performed between chick and mutant (Pax3-/GFP+) mouse embryos.</p> <p>We analyze the myogenic commitment of somites formed at the experimental zones. The expression level of Wnt11, MyoD, Sim1, Bmp4, Msx1, Pax3, Wnt1, Wnt3a, and Wnt7a is investigated.</p>

PW29-362	<p><b>COMPUTATIONAL PREDICTION OF TRANSCRIPTION FACTORS INVOLVED IN MYOGENIC DIFFERENTIATION AND THEIR BINDING SITES.</b>  HESTAND MS<sup>1</sup>, VAN GALEN M<sup>1</sup>, VILLERIUS MP<sup>1</sup>, VAN OMMEN GJB<sup>1</sup>, DEN DUNNEN JT<sup>1</sup>, 'T HOEN PAC<sup>1</sup>  (1) Center for Human and Clinical Genetics, Leiden University Medical Center, Leiden, THE NETHERLANDS.</p>
To contact the author:: M.S.Hestand@lumc.nl.	<p>The computational identification of transcription factor binding sites is difficult due to their small size, resulting in large numbers of false positives and negatives in current approaches. Two computational methods to reduce false positives are to look for over-representation of transcription factor binding sites in a set of similarly regulated promoters or look for conservation in orthologous promoter alignments.</p> <p>We have developed a novel tool titled CORE_TF (Conserved and Over-REpresented Transcription Factors) that identifies common transcription factor binding sites in promoters of co-regulated genes. To improve upon existing binding site predictions, the tool searches for TransFac<sup>R</sup> matrices that are over-represented compared to a random set of promoters and identifies cross-species conservation in the predicted transcription factor binding sites. The algorithm has been evaluated using expression array data from several literature and in house studies on myogenic differentiation.</p> <p>CORE_TF is accessible as a web interface at <a href="http://www.LGTC.nl/CORE_TF">www.LGTC.nl/CORE_TF</a>. It provides a table of over-represented transcription factor binding sites in a user-defined set of promoters and a graphical view on evolutionary conserved transcription factor binding sites. In our myogenic test data sets it successfully predicts target transcription factors and their binding sites. Binding sites for the transcription factors MAF, NF-1, and Runx2 were significantly over-represented in the upregulated genes from all microarray studies analyzed. In addition to other known muscle-related transcription factors, we have predicted the involvement of transcription factors not previously known to function in myogenesis. We are in the process of verifying results with high throughput sequencing of chromatin-immunoprecipitated samples. The combination of <i>in silico</i> and empirical approaches will assist in the identification of transcription factors with a role in the regulation of myogenic differentiation and associated with myogenic defects seen in many neuromuscular disorders.</p>

PW29-363	<p><b>MYOGENIC DIFFERENTIATION OF HUMAN EMBRYOID BODIES (EBS)</b>  PETERSSON SJ<sup>1</sup>, FRANSEN U<sup>2</sup>, JENSEN CH<sup>3</sup>, KASSEM M<sup>3</sup>, SCHROEDER HD<sup>1</sup>  (1) Department of Clinical Pathology, Institute of Clinical Research, University of Southern Denmark, Odense, DENMARK. (2) Immunology and Microbiology, Institute of Medical Biology, University of Southern Denmark, Odense, DENMARK. (3) Medical Biotechnology Center, Institute of Medical Biology, University of Southern Denmark, Odense, DENMARK.</p>
To contact the author:: sjuhl@health.sdu.dk.	<p>Human EBs were co-cultured with C2C12 mouse myoblasts to investigate if the myogenic cells could direct the embryonic stem cells into myogenic differentiation. In vitro EB differentiation is disorganized and frequently varies from one EB to another in the same culture. C2C12 myoblasts are cultured on Extra Cellular Matrix-coated coverslips with DMEM containing 10%FCS and 1%P/S. The EBs are then added directly to an established C2C12 culture. The EBs adhere to the C2C12 cells and spread out. The co-cultures are kept for one week during which the C2C12 cells are induced to differentiate by changing to DMEM supplemented with 2%HS.</p> <p>Few myotubes of human origin were found in the marginal zone of the EB, visualized by ICC using human specific anti-nuclei antibody (MAB1281). The myotubes produce Desmin, NCAM, and Myogenin. Double-staining with human specific CD56 (Leu19) and MAB1281 was used to distinguish myotubes formed by fusion of mouse myoblasts and human stem cells from myotubes of genuine human origin.</p> <p>EBs were prior to the co-culture experiments kept in suspension culture for 20 days. During this period they were induced to differentiate with and without ActivinB. No difference in myotube formation was detected between induced and control EBs. The myogenic enrichment seemed to be dependent on a direct contact with live, proliferating C2C12 mouse myoblasts cells. Culturing EBs alone on ECM-coated surfaces in conditioned medium from C2C12 myoblasts did not result in myotube formation, neither did co-culture with fixated C2C12 cells with and without conditioned medium. Co-culturing of Dil-incorporated EBs and human myoblasts resulted in formation of very few myotubes.</p> <p>For embryonic stem cells to achieve their clinical potential as a source for cell-based technologies, methods for generating large quantities of the desired end products must be developed. This is not possible solely with the methods demonstrated in this study.</p>

PW29-364	<p><b><u>DISSECTING THE ROLE OF PITX2C DURING MYOGENESIS</u></b>  FRANCO D<sup>1</sup>, VELASCO E<sup>1</sup>, MARTINEZ S<sup>1</sup>, LYONS G<sup>2</sup>, NAVARRO F<sup>1</sup>, ARANEGA A<sup>1</sup>  (1) University of Jaen, Jaen, SPAIN. (2) University of Wisconsin, Madison, USA.</p>
To contact the author:: dfranco@ujaen.es.	<p>Pitx2 is a member of the bicoid family of homeodomain transcription factors that plays a relevant role in morphogenesis. Pitx2 expression has been detected in many tissues during development, including myotomes as well as in migrating myoblasts. Its expression is also maintained in Pax3 positive cells that have completed migration at the proximal limb bud. We have previously documented that overexpression Pitx2c – isoform in undifferentiated myoblasts (Sol8 myogenic cell line) resulted in upregulation of cell cycle genes (c-myc, cyclinD1 and D2) while it arrests differentiation into mature myotubes by upregulating Pax3 and downregulating myogenic transcription factors such as MyoD and myogenin. These observations indicate that c-isoform of Pitx2 plays a pivotal role modulating proliferation vs differentiation during skeletal myogenesis. We report herein that transient transfections leading to overexpression Pitx2c in Sol8 myoblasts demonstrate that the Pitx2c effects in this cell line are dose-dependent. Therefore, we have determined at which doses of transfection Pitx2c began to induce changes in cell phenotype, inhibiting myocyte differentiation and myotube formation. Real-time PCR analysis after Pitx2c-transfection reveals that cell cycle genes (Cyclin D1 and Cyclin D2) as well as Pax3 upregulation coincides with induced changes in cell phenotype, whereas myogenic regulatory factors (MyoD, Myogenin) becomes down-regulated at low doses of Pitx2c-transfection before the onset of changes in the phenotype. These data suggests that regulation of genes involved in the maintenance of proliferative stages in myoblast and genes involved in the onset of differentiation (MyoD and Myogenin) require different Pitx2c doses. Interestingly, we found Pax7 down-regulation after low doses of Pitx2c-transfection coinciding with MyoD and Myogenin down-regulation suggesting that Pitx2 could play a role modulating Pax3/7 function in adult satellite cells.</p>

PW29-365	<p><b>EXPRESSION PROFILE OF MUSCLE PROGENITOR MATURATION DURING MOUSE DEVELOPMENT AND ADULTHOOD</b>          ROCHAT A<sup>1</sup>, ALONSO S<sup>1</sup>, MORAIS J<sup>1</sup>, RELAIX F<sup>1</sup>          (1) Mouse Molecular Genetics group, UMR S 787 - Groupe Myologie, INSERM - UPMC-Paris VI – Institut de Myologie, Faculté de Médecine Pitié-Salpêtrière, Paris, FRANCE.</p>
To contact the author:: anne_rochat@hotmail.com.	<p>Growth and repair of adult skeletal muscle is achieved by a population of progenitor/stem cells, the satellite cells. Satellite cells are derived from a fetal population of <i>Pax3</i>-expressing progenitor cells providing successive waves of myogenic cells to sustain muscle growth during development</p> <p>We are interested in the identification of genes involved in the acquisition of stem-cell properties by the fetal progenitor when they progress into the post-natal satellite cell lineage.</p> <p>We took advantage of the <i>Pax3<sup>GFP/+</sup></i> mice, in which GFP labels the muscle progenitor cells (Relaix et al., 2005), to perform an expression profiling of muscle progenitor cells from early embryonic development to adulthood. RNAs of the FACS-sorted GFP-positive cells were subjected to a microarray analysis. By comparing different time points, it revealed the progressive modifications of the progenitor cell transcriptome over time. Strikingly, more than 3000 transcripts are regulated during the transition from primary to secondary myogenesis while about 1000 transcripts are regulated at later time points, suggesting that the adult muscle progenitor cells are molecularly defined at the fetal stage.</p> <p>In addition to the analysis and validation of the screen, we will present preliminary work on new factors which might be involved in satellite cells formation.</p> <p><b>Relaix, F., Rocancourt, D., Mansouri, A. and Buckingham, M. (2005). A Pax3/Pax7-dependent population of skeletal muscle progenitor cells. <i>Nature</i>, 435, 948-953.</b></p>

PW29-366	<p><b>EXPRESSION OF VOLTAGE ACTIVATED CHLORIDE CHANNEL CLC-1 DURING SKELETAL MUSCLE SATELLITE CELLS DIFFERENTIATION</b></p> <p>ROSAS-SÁNCHEZ F<sup>1</sup>, MARTÍNEZ-TORRES A<sup>2</sup></p> <p>(1) Instituto de Neurobiología, Universidad Nacional Autónoma de México, Querétaro, MEXICO. (2) Instituto de Neurobiología, Universidad Nacional Autónoma de México, Querétaro, MEXICO.</p>
<p>To contact the author:: ferrossanchez@yahoo.com.mx.</p>	<p>Muscle satellite cells (SMCs) are quiescent precursors of myoblast with specific localization and cytology properties (Mauro, 1961; Muir et al., 1965; Gibson et al., 1982; Schmalbruch y Hellhammer., 1977). SMCs are able to proliferate, differentiate in myoblast and fusion to create new myofibers in response to damage or muscle disease (Bischoff, 1994; Zammit et al., 2004).</p> <p>Some myopathies are characterized by skeletal muscle degeneration as consequence of genetic mutations, particularly in one chloride channel of the CIC family: CIC-1 (Koch et al., 1992), and little is known about molecular mechanism that induce the precise temporal gene expression that codified for CIC-1, a specific protein in skeletal muscle, including the differentiation processes of SMCs.</p> <p>In another hand, calcium ions play a critical rule in cellular fusion processes that occur during skeletal muscle differentiation and regeneration (Shainberg et al., 1969), evidence of that is the spontaneous intracellular calcium transient release during muscular embryogenesis (Strube at al., 2000) and during SMCs differentiation processes (Bidaud et al., 2006).</p> <p>Similar events precede the presence of CIC-1. Previous to muscle cell differentiation the proper expression of CIC-1 secures a key component for the regulation of the electrical excitability of the plasma membrane. How is this balance achieved? Thus far we don't know if the transcription of CIC-1 in the SMCs relays on the expression and activity of VDCCs or VDCC currents relays the transcription of CIC-1; furthermore it may responds to a genetically programmed pattern, and expression of myogenic or cell-specific transcription factors.</p> <p>In the present study we will determine: a) the time expression of CIC-1 and VDCCs, <i>T-type</i> and <i>L-Type</i>, in mammalian SMCs after muscle injury, b) the correlation between the expression of CIC-1, VDCCs and myogenic markers during the differentiation process of SMCs and c) if CIC-1 expression affect VDCCs expression pattern or viceversa.</p>

PW29-367	<p><b>EFFECTS OF WNT4 ON THE MYOGENIC DIFFERENTIATION</b>  BERNARDI H<sup>1</sup>, GAY S<sup>1</sup>, FEDON Y<sup>1</sup>, BOLZEC T<sup>1</sup>, BACOU F<sup>1</sup>  (1) INRA, UMR 866, Laboratoire de Différenciation Cellulaire et Croissance, Montpellier, FRANCE.</p>
To contact the author:: bernardi@supagro.inra.fr	<p>The molecular signals that regulate satellite cell function remain largely obscure. However, it was recently demonstrated that Wnts participate in the temporal control of satellite cell expansion versus differentiation during adult muscle regeneration (Brack et al, 2008). Thus, differentiation of myoblasts <i>in vitro</i> and <i>in vivo</i> is correlated with an upregulation of canonical Wnt signaling. Furthermore, ectopic Wnt induces premature muscle differentiation whereas inhibition of Wnt signaling interferes with muscle differentiation. In this context, the fact that myostatin -a member of the TGF-<math>\beta</math> superfamily that specifically regulates muscle mass- was shown to implicate Wnt4 signaling in postnatal skeletal muscle hypertrophy is of the utmost importance (Steelman et al, 2006). This was corroborated by Takata et al, (2007) showing the involvement of Wnt4 signaling during myogenic proliferation and differentiation of skeletal muscle. In this context, we first established by SQ-PCR that a limited number of Wnts was expressed during proliferation and differentiation of C2C12 myoblasts and satellite cells (SC). Amongst the 19 Wnts examined, we noticed that only the expression of Wnt4 was lacking during proliferation and was highly induced during differentiation of both cell types. The aim of this study was thus to characterize the role of Wnt4 protein in muscle homeostasis. The effects of Wnt4 on myogenic differentiation were examined by modulating the expression level of this factor. We showed that over-expression of Wnt4 in proliferative state by transient or stable transfection was responsible for spontaneous differentiation (C2C12) or an important myotube hypertrophy (SC). Conversely, inhibition of Wnt4 expression by siRNA silencing on SC led to an atrophy of differentiated myotubes (myotube areas correspond to 40% of siRNA luciferase controls) and a decrease in fusion index (24%). Treatment of C2C12 myoblasts by Wnt4 siRNA induced a strong inhibition of differentiation associated with a decrease in the expression of Myf5.</p>

PW29-368	<p><b><u>WNT NON-CANNONICAL SIGNALING REGULATES STEM CELL SELF-RENEWAL WITHIN THE MUSCLE SATELLITE CELL NICHE</u></b>  LE GRAND F<sup>1</sup>, JONES A<sup>1</sup>, SCIMÉ A<sup>1</sup>, RUDNICKI M<sup>1</sup>  (1) Ottawa Health Research Institute, Ottawa, CANADA.</p>
<p>To contact the author::  fabienlegrand@gmail.com.</p>	<p>Skeletal muscle growth and regeneration are attributed to satellite cells which are myogenic cells lying between the myofiber sarcolemma and basal lamina. Recent findings in our lab indicated that the satellite cell pool can be divided in 2 lineages on the basis of the activation of either Myf5-LacZ or Myf5-Cre reporter alleles. Myf5+ satellite cells represent a committed progenitor whereas the Myf5- sub-population possesses repopulating ability. We designed a FACS strategy for the specific selection of both satellite cell lineages and subsequently performed genetic differential screening utilizing suppression subtractive analysis (SSH). Adult satellite cells were FACS-sorted from muscle by <math>\alpha 7</math> integrin and CD34 immunoreactivity. Targeted satellite cell populations were then separated on the basis of the expression of the Myf5-YFP reporter. Real-Time PCR analysis showed both populations express numerous satellite cell-specific genes at a similar level. Selected YFP- and YFP+ cells were able to proliferate <i>in vitro</i> and give rise to functional cycling myoblasts and differentiated myotubes. RNA was extracted from quiescent cells and their proliferating progeny and subjected to SSH to identify differentially expressed transcripts in proliferating myoblasts and YFP+ and YFP- satellite cells. This approach allowed us to identify numerous genes specifically expressed in quiescent cells, and showed parallels with embryonic myogenic progenitors. Comparison of YFP- and YFP+ satellite cells demonstrated that more than 30 genes are differentially expressed between these two populations. Further analysis of specific cDNAs suggested that the Notch pathway, as well as Wnt non-canonical signaling, are involved in satellite stem cells maintenance. We investigated the impact, <i>in vitro</i> and <i>in vivo</i>, of the modulation of these molecular pathways on the commitment of muscle stem cells and demonstrated that Wnt7a signaling controls symmetric division and self-renewal in the satellite niche. Our findings may help design new therapies to alleviate muscular dystrophy by enhancing muscle regeneration.</p>

PW29-369	<p><b><u>IMPLICATION OF PITX GENES DURING SKELETAL MUSCLE DEVELOPMENT</u></b>  L'HONORÉ A<sup>1</sup>, COULON V<sup>1</sup>, DROUIN J<sup>1</sup>  (1) Institut de Recherches Cliniques de Montreal, Montreal, CANADA.</p>
To contact the author:: lhonora@ircm.qc.ca.	<p>The myogenic program of cell differentiation is controlled by different groups of transcription factors acting during muscle development. We reported the expression of Pitx transcription factors throughout muscle development. The earliest member of this homeobox subfamily to be expressed in myogenesis is Pitx2 that is expressed in dermomyotome delaminating cells, and in proliferative muscle progenitors located within myotome and limb muscles masses. Pitx3 expression is then concomitant with myoblast determination of progenitors. While Pitx2 expression decreases in differentiated cells, Pitx3 is maintained until birth in all skeletal muscles of the body and limbs. We first analyzed Pitx3 function by use of a natural mouse mutant of this gene. Indeed, the <i>aphakia</i> (<i>ak</i>) mouse was shown to have severely deficient <i>Pitx3</i> expression in midbrain and eyes, and thus constitute a model of loss-of-<i>Pitx3</i> function. However, we found unaltered <i>Pitx3</i> expression in muscles of <i>ak</i> mice. This led us to identify and characterize in transgenic mice a muscle-specific <i>Pitx3</i> promoter that is intact in the <i>ak</i> mice. To achieve Pitx3 loss-of-function in muscle, we generated a complete Pitx3 knockout mouse. Pitx3 deficiency does not significantly perturb muscle development but results in complete compensation through maintenance of Pitx2 expression in all skeletal muscles until birth. These experiments indicate that the level of Pitx mRNAs is maintained through compensatory mechanisms in skeletal muscles and this suggests that Pitx2 and Pitx3 may redundantly serve critical functions in muscle development. To assess these functions, we are currently investigating Pitx2 and Pitx3 complete and muscle-specific double knock-out mice phenotype.</p>

PW29-370	<p><b><u>PROTEOME DYNAMICS OF BOVINE MUSCLE DURING FOETAL MYOGENESIS</u></b>          CHAZE T<sup>1</sup>, MEUNIER B<sup>1</sup>, CHAMBON C<sup>2</sup>, JURIE C<sup>1</sup>, PICARD B<sup>1</sup>          (1) INRA, UR 1213, Unité de Recherche sur les Herbivores, Saint Genès-Champanelle, FRANCE. (2) UR 370 Plateforme Protéomique du Centre INRA de Clermont Ferrand Theix, Saint Genès-Champanelle, FRANCE.</p>
To contact the author:: bpicard@clermont.inra.fr	<p>Pre-natal period is crucial for muscle development in large species such as <i>Bos taurus</i> since the most part of differentiation process is completed at birth. Previous bovine studies displayed developmental stages representative of particular myogenic events across the nine months of gestation. Production of bovine foetuses at these specific stages is really interesting to begin multidisciplinary studies using <i>in vivo</i> biological material. Beside classical methods (histology, immunodetection), proteomic is a method of choice that can give access to the entire genome expression during myogenesis. Two-dimensional electrophoresis and mass spectrometry identification of proteins were conducted to display the muscular proteome dynamics through bovine myogenesis. This study allowed the identification of 250 proteins grouped in different expression profiles using Principal Component Analysis and Hierarchical Clustering Analysis. Most part of proteins referred to contractile apparatus, energy metabolism, cytoskeleton component and cell cycle actors. Early myogenic events were strongly characterized first by a large number of differentially expressed proteins and second by a large class of proteins involved in the control of the balance proliferation /apoptosis. On the other hand, the last third of pre-natal life was mainly characterized by i) few differentially expressed proteins, ii) many isoforms changes of contractile and metabolic proteins and iii) a more important number of common proteins across the last developmental stages.</p> <p>This study allows a wide vision of proteome dynamics across foetal myogenesis and is in agreement with previous genomic approach conducted with transcriptomic tool on total mRNA. These results are of fundamental interest to myogenesis in general, and could be important for human biology since bovine and human share a lot of common features during skeletal myogenesis from the gestation time to the course of the proliferation of different myoblasts generations. Studies based on bovine model could potentially conduct to new insights for human knowledge.</p>

PW29-371	<p><b><u>SIX1 AND SIX4 GENES ARE REQUIRED TO ACTIVATE THE FAST-TYPE MUSCLE GENE PROGRAM IN THE MOUSE EMBRYO.</u></b>  NIRO C<sup>1</sup>, DEMIGNON J<sup>1</sup>, GRIFONE R<sup>2</sup>, GIORDANI J<sup>1</sup>, MAIRE P<sup>1</sup>  (1) INSTITUT COCHIN INSERM U567 CNRS UMR8104, PARIS, FRANCE. (2)  Developmental Biology Institute of Marseilles UMR 6216, MARSEILLES, FRANCE.</p>
To contact the author:: niro@cochin.inserm.fr.	<p>The mechanisms that participate to the adult muscle slow fibre phenotype have been well characterized. These mechanisms emphasize resting calcium concentration as a key cofactor relaying slow motoneuron firing and controlling the activity of several signalling pathways (calcineurin, CamK) that activate different transcription factors (NFAT, MEF2, PGC1<math>\alpha</math>). On the contrary, the mechanisms that govern the genesis of muscle fibre heterogeneity during development are still unresolved in mammals. Several signalling pathways have been discovered in zebrafish that trigger slow (SHH/Blipm1) and fast-type (FGF8/Pbx-Myod) muscle lineages. However their relevance is not established in other species. We have previously shown that the Six1 homeoprotein is specifically enriched in muscle nuclei of fast-type muscle fibres and that forced expression of Six1 and its Eya1 cofactor in the soleus converts its slow oxidative phenotype to a fast glycolytic one.</p> <p>We now show that Six proteins are responsible for the activation of the fast muscle program during the earliest steps of muscle development in the mouse embryo : fast-type muscle genes expression is not detected by in situ hybridization in <i>six1</i><sup>-/-</sup><i>six4</i><sup>-/-</sup> embryos at E10.5 whereas slow muscle gene isoforms are still expressed in the remaining myocytes. While several muscle defects have been already characterized in <i>six1</i><sup>-/-</sup> embryos, but not in <i>six4</i><sup>-/-</sup>, we have shown an aggravation of the phenotype in <i>six1</i><sup>-/-</sup><i>six4</i><sup>-/-</sup> embryos, suggesting some compensatory functions between these two genes. We now show that Six1 null embryos are not impaired in their fast-type muscle genes activation. Thus activation of the fast muscle program in the primary myotome can be achieved with Six4 homeoprotein alone, showing that Six1 has no intrinsic properties as compared with Six4 in regard of this induction. We also provide evidence that the known Eya cofactors of Six homeoproteins are dispensable to achieve fast-type muscle genes specific activation in the mouse E10 embryo.</p>

