PW 39:

Young investigator Symposium

Lectures

(Tuesday May, 27 – Afternoon)

PW39-488	**MUSCLE PERFUSION AND BIOENERGETICS MEASURED IN VIVO IN THE MOUSE BY FUNCTIONAL 1H-NMR IMAGING AND 31P-NMR SPECTROSCOPY.** BALIGAND C[1], WARY C[1], MÉNARD J[1], BERTOLDI D[1], GIACOMINI E[1], CARLIER PG[1] (1) Laboratoire de RMN AIM - CEA, Institut de Myologie, Paris, FRANCE.
To contact the author:: c.baligand@myologie.chups.jussieu.fr.	^{1}H-NMR imaging combined with arterial spin labeling (ASL) provides dynamic, quantitative and non invasive measurements of muscle perfusion as well as blood oxygenation level dependent (BOLD) contrast. In Parallel, ^{31}P-NMRS has long been used to investigate muscle exercise metabolism but remains challenging in mouse because of the small size of the animal. The cross analysis of these three parameters is rarely explored, however valuable for an accurate characterization of muscular diseases. In this work, a complete experimental set-up has been developed to simultaneously and non-invasively assess perfusion, BOLD and phosphorus metabolites in mouse, in response to electrical stimulation. Experiments were performed inside a Bruker *Biospec* 4T NMR system using custom-built coils. Muscle contractions were induced with an electrostimulator through subcutaneous electrodes. In order to compensate for minimal ^{31}P signal, we explored the possibility to sum the results of 10 consecutive exercises. C57/bl6 mice were repeatedly subjected to a "rest(30s)- exercise(30s)- recovery(9min)" protocol. Perfusion/BOLD and phosphocreatine content (PCr) were monitored with a time resolution of 9s and 2s, respectively. Time courses of rephosphorylation of PCr were fitted with a single exponential function. Exercise systematically resulted in a total consumption of PCr. Owing to high time resolution, we could measure the subsequent initial rate of PCr recovery, a direct estimate of mitochondrial oxidative capacity, to have a time constant τPCr= 77 +/- 16s. Perfusion was 6.6 +/- 3.4 ml.min^{-1}.100g^{-1} at rest and rose to 141 +/- 13 ml.min^{-1}.100g^{-1} immediately after the end of the exercise. We also observed a transient increase in BOLD signal. This is the first report of a totally non-invasive assessment of mouse skeletal muscle function in vivo by combined NMR imaging and spectroscopy. This tool offers new perspectives to study muscle pathology in mice models and monitor longitudinal effect of therapeutic protocols.

PW39-489	**EXPRESSION OF SELENOPROTEIN N IN MICE DURING DEVELOPMENT** CASTETS P[1], MAUGENRE S[1], GARTIOUX C[1], REDERSTORFF M[2], LESCURE A[2], KROL A[2], TAJBAKHSH S[3], ALLAMAND V[1], GUICHENEY P[1] (1) INSERM UMR582, UPMC Paris 6, Institut de Myologie, IFR14, Paris, FRANCE. (2) UPR 9002, CNRS, IBMC, Strasbourg, FRANCE. (3) CNRS, URA 2578, Institut Pasteur, Paris, FRANCE.
To contact the author:: p.castets@institut-myologie.org.	SEPN1-related myopathy is a rare disorder characterized by axial muscle weakness, scoliosis and respiratory failure, and caused by mutations in the *SEPN1* gene, encoding selenoprotein N (SelN). This entity gathers four autosomal recessive muscular pathologies with molecular, clinical and morphological overlap: Rigid Spine Muscular Dystrophy, the classical form of Multi-minicore Disease, rare cases of Desmin–Related Myopathy with Mallory Body–like Inclusions and Congenital Fibre Type Disproportion. SelN is a glycoprotein of as yet unknown function, localized in the membrane of the endoplasmic reticulum. As all selenoproteins, SelN is characterized by a specific selenocysteine residue in its peptidic sequence. The clinical features observed in patients and the defects in muscle organization seen in zebrafish mutants, led us to hypothesize that SelN may play a role during muscle development and/or maintenance. To precise *Sepn1* expression during murine development and due to a lack of robust antibodies against SelN, we used RNA-based approaches: quantitative RT-PCR (qRT-PCR), performed on cDNA obtained from isolated adult and embryonic tissues, and whole mount *in situ* hybridization. We demonstrated that *Sepn1* is expressed early during embryogenesis, with a strong expression already detected at E9. By qRT-PCR, we showed that this expression increases until E12 and then markedly decreases. Between E15 and E18, its decline is also detected in most isolated tissues. After birth, a strong reduction is observed with age in most tissues, leading to barely detectable levels at 6 weeks. In some rare tissues, such as liver, *Sepn1* expression was not detectable, neither in embryos nor after birth. These results indicate that SelN is developmentally regulated in mice and correlate with data obtained in human that showed it is strongly down regulated during myoblasts differentiation. We are currently investigating muscle embryonic development in SelN deficient mice by characterizing the expression of myogenic factors in Sepn1-/- embryos.

PW39-490

TRANSDUCTION OF LOWER MOTOR NEURONS USING PERIPHERAL INJECTION OF AAV VECTORS

DUQUE S[1], JOUSSEMET B[2], RIVIÈRE C[3], MARAIS T[1], DUBREIL L[4], DOUAR AM[3], MOULLIER P[2], COLLE MA[4], BARKATS M[1]
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Gene therapy for Central Nervous System (CNS) disorders is impeded by the Blood Brain Barrier (BBB), a physical barrier preventing the entry of viral vectors. For motor neuron diseases (e.g. Spinal Muscular Atrophy (SMA) and Amyotrophic Lateral Sclerosis (ALS)), this problem has been previously circumvented by injecting viral vectors directly into the spinal cord. This approach resulted only in the transduction of cells close to the injection site. The development of strategies less invasive and allowing a larger dispersion of viral vectors and/or therapeutic proteins in the CNS is therefore required for future clinical application.

Here, we describe a new gene transfer method that allows efficient transduction of lower motor neurons after peripheral injection of recombinant AAV vectors. We first injected single strand (ss) and self-complementary (sc) AAV vectors of serotype 1 and 9 intraperitoneally, intramuscularly or intravenously in neonatal and adult C57Bl6 mice and analyzed transgene expression in the CNS. We found that both recombinant ss- and scAAV9 vectors targeted epithelial cells of both the choroids plexus and the ependyma, as well as neural cells including motor neurons. In mice, the most impressive transduction was observed after intravenous administration of scAAV9 vectors. Futhermore, the ability of scAAV9 to efficiently cross the BBB and transduce motor neurons was confirmed in a feline model of SMA. In these animals, intravenous vector injections led to a transduction of 38% of motor neurons.

In conclusion, this study describes the first efficient and non invasive procedure that allows a global transgene delivery to the spinal cord *via* a single intravenous injection. This new gene therapy tool offers new hope for the treatment of motor neuron diseases.

PW39-491	**USE OF NONSENSE-MEDIATED MRNA DECAY (NMD) INHIBITORS TO CORRECT GENETIC PATHOLOGIES** DURAND S[1], TAZI J[1], LEJEUNE F[2] (1) IGMM-CNRS-UMR5535, Montpellier, FRANCE. (2) Institut Pasteur de Lille, Lille, FRANCE.
To contact the author:: sebastien.durand@igmm.cnrs.fr.	Nonsense-Mediated mRNA Decay (NMD) is an mRNA quality control process that degrades mRNA containing premature termination codons (PTC) in order to avoid the production of truncated proteins with potential deleterious effects for cells. One third of inherited and acquired diseases are due to nonsense mutations that elicit NMD. In some cases such as Duchenne Muscular Dystrophies (DMD), NMD could be detrimental to the production of truncated proteins that has kept the activity of wild-type proteins. Consequently, an interesting challenge would be to inhibit NMD in order to restore truncated protein whose expression could lead to clinical rescue. We have screened a small chemical library to find inhibitors of NMD. We have identified the first chemical inhibitor that blocks efficiently and specifically NMD (J. Cell Biol 2007 sept 24; 178(7):1145-1160). We have shown that this NMD inhibitor called NMDI 1 (Nonsense-mediated mRNA Decay Inhibitor 1) disrupts the interaction between hSMG5 and the NMD factor hUPF1 thereby leading to the accumulation of hyperphosphorylated isoforms of hUPF1 in cytoplasmic granules so called processing-bodies (P-Bodies). NMDI 1 stabilizes PTC-containing mRNAs in P-Bodies and could give rise to truncated proteins. To address the question of the therapeutic potential of NMDI 1, we have used cell lines from DMD patients but also different mouse models of NMD: the mdx mice that contain a PTC in the exon 23 of the dystrophin gene and the KIM mice that carry a PTC in the exon 3 of the gene coding the μ opioïd receptor. Preliminary results have shown that NMDI 1 is able to stabilize dystrophin mRNA in patient cell line and lead to truncated protein expression rescue when NMDI 1 is directly injected in mice.

PW39-492	**MS2PH-DB : TOWARD THE INTEGRATION OF PROTEIN STRUCTURAL BEHAVIOUR TO STUDY PHENOTYPIC IMPACTS OF MUTATIONS** FRIEDRICH A[1], GARNIER N[2], BLANDIN G[3], BETTLER E[2], RICHARD I[3], MOULINIER L[1], POCH O[1] (1) IGBMC, Illkirch, FRANCE. (2) IBCP, Lyon, FRANCE. (3) Généthon, Evry, FRANCE.
To contact the author:: friedric@igbmc.u-strasbg.fr.	The MS2PH project (from Structural Mutation to Human Pathologies Phenotypes) aims at investigating how mutations impact protein structures and to which extent this change could affect pathological phenotypes. This project is part of the Decrypthon program (www.decrypthon.fr) where we have implemented the MS2PH-db relational database (http://ms2phdb-pbil.ibcp.fr/). This database focuses on 1915 proteins implicated in human monogenic diseases, including 70 proteins directly implicated in various muscular dystrophies, and offers access to mined, predicted and pre-computed data. MS2PH-db provides instant access to three types of data for each entry protein: - an evolutionary overview through pre-computed hierarchized multiple alignments of complete sequences, - a structural view through an automatically generated three-dimensional model of the protein, - an additional layer of information composed of structural and functional annotations, as well as mutations and phenotypic data related to the pathology. These three views are interconnected through a user-friendly graphical interface which allows an interactive approach to study the combination of structural, functional and clinical data within the framework of the evolutionary relevance of the sequence information. Using this tool, we have studied the distribution of all mapped mutations within the 1915 MS2PH proteins with respect to known functional domains. We observed that roughly 78% of the mutated residues associated with a phenotypical impact are located in close or medium proximity of a conserved residue. In the frame of the newly developed Decrypthon Data Center, we are currently integrating the structural and evolutionary information with interactomic data, using protein interactions data provided by complementary sources: experimental interaction data generated in the Muscular Interactome project, functional and physical interactions mined from the STRING database and interacting interface predictions. The preliminary results obtained with this integrative approach are showing the way to the establishment of a system dedicated to the prediction of the link between mutations and clinical phenotypes.

PW39-493	**TROPISM CHARACTERIZATION OF DIFFERENT VIRAL VECTORS IN THE PERIPHERAL NERVOUS SYSTEM** HOMS J[1], ARIZA L[1], RODRÍGUEZ E[1], CHILLÓN M[1], CHILLÓN M[2], BOSCH A[1] (1) Centre of Animal Biotechnology and Gene Therapy (CBATEG) and Department of Biochemistry and Molecular Biology, Universitat Autònoma de Barcelona, Barcelona, SPAIN. (2) Institució Catalana de Recerca i Estudis Avançats (ICREA), Barcelona, SPAIN.
To contact the author:: Judit.Homs@uab.cat.	Central nervous system transduction has been extensively studied with a wide variety of viral vectors. However, peripheral nervous system transduction has not brought that much attention to researchers so far, despite the importance of acquired and inherited neuropathies, neuromuscular diseases or pain treatment, among other pathologies. Thus, we have characterized the tropism and transduction efficiency of different AAV pseudotypes in primary and established Schwann cell lines as well as *in vivo*, through sciatic nerve injection in the mouse. Among the AAV pseudotypes tested, AAV2/8 transduced Schwann cells more efficiently, both *in vitro* and *in vivo*. On the other hand, AAV2/2 infected preferentially sensory neurons and AAV2/1 transduced both Schwann cells and neurons. Expression of marker genes coded by the different vectors was still present 10 weeks after administration, the overall duration of the experiment. We also analyzed the generation of neutralizing antibodies against AAVs in the infected mice. Antibody titers were higher against AAV1 than against AAV2 or AAV8. Indeed, animals injected with AAV8 showed the lower titers of neutralizing antibodies against this serotype, correlating with higher expression overtime. These results suggest that AAV8 may be a suitable tool for gene therapy treatment of diseases affecting Schwann cells. To improve transduction of peripheral neurons, we infected organotypic cultures of murine dorsal root ganglia with different serotypes of AAVs and human and canine adenoviruses. These vectors were also tested *in vivo* by intrathecal administration to the lumbar region of mice. We observed high levels of expression in sensory neurons with AAV2/1 and with human Ad5. We are currently evaluating efficiency of canine adenovirus and human Ad40 serotype in targeting sensory and motoneurons. In the near future, helper-dependent adenovirus will be assayed with therapeutic genes in a mouse model of disease. Financed by AFM (#12277; AFM2007/12763AE), ISCIII (PI051705) and Generalitat de Catalunya (2006FI00762; 2004FI00970).

PW39-494	**SKELETAL MUSCLE CELL FATE DECISIONS IN THE EMBRYO AND THE ROLE OF NUMB** JORY A[1], TAJBAKHSH S[1] (1) Stem cells and development, Pasteur Institute, Paris, FRANCE.
To contact the author:: ajory@pasteur.fr.	Although much is known about skeletal muscle biology, we have only recently begun to appreciate how stem and progenitor cells establish this tissue through the action of key regulatory genes. In the mouse embryo, the dermomyotome (DM) harbors stem cells for multiple cell lignages including muscle. The DM is the epithelium of the somite which assures a continuous source of muscle progenitor cells (MPCs) from the stem cells expressing *Pax3* and *Pax7*. Muscle progenitors generate myoblasts which differentiate to form the first skeletal mass in the somite, the myotome. To study self-renewal and differentiation of MPCs, we are investigating self-renewal via asymmetric divisions. The cell fate determinant Numb has been implicated in asymmetric division and involved in binary cell fate choices via Notch inhibition in *Drosophila*. However, the mechanisms and the role of Numb involved in generating cell diversity in vertebrates remains unclear. To further assess the role of Numb in driving cell fate in the mouse dermomyotome and in establishing the myotome, several transgenics were made to study the effects of Numb-GFP over-expression in the somite using the *epaxial enhancer* of *Myf5*. First we show that these transgenics do drive NumbGFP over-expression in most of the stem cells of the dorsal somite. Unexpectedly this strategy revealed that the overexpression of Numb-GFP in the dorsal dermomyotome only very mildly affects cell fate decisions and does not seem to have an effect on Notch signalling in this context. We do not observe an overt increase of muscle differentiation in the dorsal somite. Thus, we believe that Numb and Notch act in particular cell states in lineage progression. Interestingly, this strategy revealed a novel role for Numb in somite patterning, in the epithelial organisation of the dermomyotome, as well as in myofiber organisation in the underlying myotome.

PW39-495	**ANCHORING OF ACETYLCHOLINESTERASE IN MEMBRANES BY PRIMA** NOUREDDINE H[1], CARVALHO S[1], SCHMITT C[1], MASSOULIE J[1], BON S[1] (1) Laboratoire de Neurobiologie, CNRS UMR 8544, Ecole Normale Supérieure, Paris, FRANCE.
To contact the author:: Hiba.noureddine@biologie.ens.fr.	The nervous tissue and muscles of mammals express the T splice variant of acetylcholinesterase ($ACHE_T$), characterized by its 40-residues C-terminal peptide (t peptide), which allows its association with anchoring proteins, the collagen ColQ and the transmembrane protein PRiMA, producing respectively collagen-tailed forms and membrane-bound tetramers. These interactions are important since they condition the functional anchoring of AChE in cholinergic tissues. The collagen-tailed forms are inserted in the basal lamina at neuromuscular junction, while the membrane-bound tetramers are anchored at the cell surface through the transmembrane domain of PRiMA. The membrane-bound tetramers represent the major enzyme species in the brain ; they are also expressed in muscles, where their level is regulated by exercise. The association of $AChE_T$ subunits with ColQ has been extensively studied : it is based on an tight interaction between four t peptides and a proline-rich motif, called PRAD (*"Proline-Rich Attachment Domain"*), located in the N-terminal non collagenous region of ColQ. The structure of a complexe between synthetic t and PRAD peptides has been determined by cristallography. The association of $AChE_T$ subunits with PRiMA appears similar because this transmembrane protein also contains a proline-rich motif, but there are significant differences in the number of prolines (8 in ColQ, 14 in PRiMA) and in the number and positions of cysteines that might form intercatenary disulfide bonds with the cysteine located near the C-terminus of the t peptides. Therefore, we have undertaken an analysis of the association of $AChE_T$ with PRiMA. Using deletions and point mutations in PRiMA, we defined a minimal motif in PRiMA, which could associate with $AChE_T$ subunits.

PW39-496	**CTCF AND A-TYPE LAMINS COUPLE INSULATION AND PERINUCLEAR LOCALIZATION OF THE D4Z4 SUBTELOMERIC ELEMENT IN FSHD.** OTTAVIANI A[1], RIVAL-GERVIER S[2], BOUSSOUAR A[1], FÖRSTER A[3], RONDIER D[1], BAUWENS S[1], GILSON E[1], MAGDINIER F[1] (1) Laboratoire de Biologie Moléculaire de la Cellule, Ecole Normale Supérieure de Lyon, Lyon, FRANCE. (2) UMR 1198. Biologie du développement et de la Reproduction, INRA-CNRS-ENVA, Domaine de Vilvert, Jouy-en-Josas, FRANCE. (3) Gregor Mendel-Institute of Molecular Plant Biology, Vienna, AUSTRIA.
To contact the author:: alexandre.ottaviani@ens-lyon.fr.	Both genetic and epigenetic alterations contribute to the Facio-Scapulo-Humeral Dystrophy (FSHD) linked to the reduction of a number of *D4Z4* repeated elements at the 4q35 locus. The consequence of this rearrangement remains enigmatic but deletion of these repeats to a threshold of 11 copies might epigenetically dysregulate the FSHD gene(s) in patients through position effect variegation (PEV) and our goal was to test the function of *D4Z4* on the regulation of gene silencing. We generated several constructs where different parts or different numbers of *D4Z4* were cloned downstream of a reporter gene and followed the expression of the gene over an extended time in culture. We showed that *D4Z4* is a *bona fide* insulator element protecting from PEV and able to block enhancer-promoter communication. The multivalent CTCF protein and A-type Lamins bind to *D4Z4* and participate in this insulation activity. Unlike other human telomeres, the 4q35 locus is localized at the periphery of the nucleus and might interact with components of the lamina. We showed that *D4Z4* displaces a telomere toward the nuclear periphery in the presence of CTCF and Lamins A/C suggesting that both factors coordinate the long-range chromatin organization of *D4Z4* and the tethering of a locus to a specialized nuclear compartment. We further demonstrate that insulation and perinuclear activities of *D4Z4* are lost upon multimerization of the repeat, suggesting that the *D4Z4* array at the 4q35 locus acts as a CTCF-dependent insulator in FSHD patients but not in normal individuals and might impact on the expression of the genes causing FSHD through the alteration of the 4q35 locus microenvironment.

PW39-497	**<u>UROCORTINS IMPROVE SKELETAL MUSCLE STRUCTURE AND FUNCTION OF DYSTROPHIC MDX MOUSE VIA A CYCLIC AMP/PKA DEPENDENT PATHWAY</u>** REUTENAUER J[1], DORCHIES O[1], BOITTIN FX[2], PATTHEY-VUADENS O[1], RUEGG U[1] (1) Laboratory of Pharmacology, Geneva Lausanne School of Pharmaceutical Sciences, University of Geneva, Geneva, SWITZERLAND. (2) Laboratory of Vascular Cell Physiology, Department of Zoology & Animal Biology, University of Geneva, Geneva, SWITZERLAND.
To contact the author:: julie.reutenauer@pharm.unige.ch.	Urocortins are structurally related peptides of the corticotrophin-releasing factor (CRF), which has been widely implicated in responses to physical, emotional and environmental stress. Recently, it has been reported that a CRF-receptor-2 (CRFR2) agonist increased muscle mass and slowed disease progression in the *mdx* mouse model. Here, we report on our investigations on urocortins in protecting *mdx* muscle from necrosis and on the mechanism involved. Three week-old mdx^{5Cv} mice were treated daily by sub-cutaneous injection for 2 weeks with either urocortin 1 at 300 µg/kg, urocortin 2 (30, 100 or 300 µg/kg), or vehicle. Isometric force recordings of the triceps surae (comprising soleus, plantaris and gastrocnemius muscles) showed that contraction and relaxation kinetics were shorter than in untreated mice. Moreover, the force-frequency curve was shifted to the right, suggesting a change in the calcium homeostasis or in the distribution of fibres toward a faster phenotype. Interestingly, hindlimb muscles from mice receiving urocortins displayed a higher resistance to mechanical stress and muscle mass was significantly increased. Histology demonstrated that urocortins remarkably protected diaphragm, EDL and soleus muscles from necrosis. Finally, urocortins administration lowered plasma creatine kinase levels up to 49% compared with vehicle. In addition, basal calcium influx was measured in whole dystrophic muscle (EDL and soleus) and in diaphragm strips. Urocortin 2 concentration-dependently decreased the permeability of the dystrophic muscle to calcium by 20-40%. This effect was completely abolished by the selective CRFR2 antagonist $astressin_2$-B or an inhibitor of protein kinase A (PKA) while addition of forskolin, an activator of adenylate cyclase, mimicked the effect of urocortin 2. We conclude that urocortins improve both the structure and the function of dystrophic skeletal muscle via the stimulation of a cAMP/PKA-dependent signal transduction pathway. Thus, urocortins should be considered as potential candidates to counteract the impairment of calcium homeostasis observed in Duchenne Muscular Dystrophy.