

**PW 38:
Young investigator
symposium**

Posters

PW38-465	<p><u>MORPHOMETRIC ANALYSIS REVEALED ABNORMAL COMPLEX DENDRITES IN POSTNATAL LUMBAR MOTONEURONS OF MOUSE MODEL OF AMYOTROPHIC LATERAL SCLEROSIS (ALS).</u> AMENDOLA J¹, DURAND J¹ (1) CNRS-P3M, Marseille, FRANCE.</p>
<p>To contact the author:: julien.amendola@dpm.c nrs-mrs.fr.</p>	<p>The early mechanisms leading to the progressive loss of motoneurons during ALS are still unknown. Using the <i>in vitro</i> preparation of brainstem/spinal cord isolated from wild-type and SOD1^{G85R} transgenic mice (ALS model), we have recently reported alterations of the input resistance and gain of SOD1^{G85R} lumbar motoneurons (Mns) during the second postnatal week [1]. These results suggested that the morphology of these cells could have been modified. To address this question, we have stained developing lumbar Mn with neurobiotin tracer and fully reconstructed them in 3D using the Neurolucida system. The somata of labelled Mns (SOD1^{G85R}, n=6; control (WT) n=8) were located within the same ventro-lateral region and directions of the dendrites depended on the location of the cell bodies. Dendrites mainly projected in three directions (dorsal, dorso-lateral and median). In the rostro-caudal plane, the labelled Mns extended up to 750 µm. This staining procedure revealed a highly complex morphology of SOD1^{G85R} Mns labelled from postnatal day 8 (P8) to P9 mice. The number of branching nodes in SOD1^{G85R} Mns was almost twice than that of WT. Moreover the total length and surface of dendritic trees were higher in SOD1^{G85R} Mns. The increase in complexity of the SOD1^{G85R} arborizations was due to higher occurrence of branching points at different distances from the soma.</p> <p>We had also found correlations between the total size of Mns (cell body, dendrites and axone) and several electrical properties like the input resistance, rheobase current, the gain, spike after-hyperpolarization (AHP) duration and AHP decay-time.</p> <p>In conclusion, the alterations of electrical properties of SOD1^{G85R} lumbar motoneurons are associated with morphological changes. These motoneuronal alterations took place during the period of maturation and could have important consequence on the pathophysiology of ALS.</p> <p>[1]- Bories <i>et al</i>, 2007, <i>Eur. J. Neurosci.</i> 25(2):451-9.</p>

PW38-466	<p><u>A LARGE NUCLEAR RETAINED NON-CODING RNA REGULATES SYNAPTOGENESIS.</u> BERNARD D¹, PRASANTH KV¹, SEDEL F¹, TRILLER A¹, SPECTOR DL¹, BESSIS A¹ (1) INSERM U789, Ecole Normale Supérieure, Paris, FRANCE.</p>
	<p>During physiological development of spinal cord, half of the motoneurons die few days after their generation. It has been shown that motoneurons are not committed to death but rather acquire the competence to die upon a transient microglial TNFα signalling (Sedel et al., 2004). In order to identify motoneuron molecules implicated in this process, we isolated 24 candidate genes expressed by motoneurons during the acquisition of the competence to die. One of these candidates is a non-coding RNA (ncRNA) referred to as metastasis associated lung adenocarcinoma transcript-1 (<i>Malat1</i>) (Ji et al., 2003). <i>Malat1</i> is a mammal specific 7kb nuclear ncRNA that is highly expressed in motoneurons during development and in adult. <i>Malat1</i> localizes to nuclear speckles (Lamond and Spector, 2003) and knockdown studies revealed that it is required for the proper recruitment of SF2/ASF splicing factor. In neurons, <i>Malat1</i> is detected concomitantly with synaptogenesis. Consistent with this observation, inhibition and overexpression of this ncRNA regulates positively synaptogenesis in cultured neurons, which correlates with its effect on the expression of at least two genes involved in synaptogenesis : neuroligin1 (<i>Nlgn1</i>) and synaptic cell adhesion molecule (<i>SynCAM</i>) (Chih et al., 2005 ; Biederer et al., 2002). Preliminary results now show that <i>Malat1</i> is upregulated in pathological motoneurons such as axotomized and SOD mutant motoneurons.</p>

PW38-467	<p>FUNCTIONAL CHARACTERIZATION OF A NOVEL MITOCHONDRIAL PROTEIN CAMBIER L¹, RASSAM P¹, AUFRAY C², POMIÈS P¹ (1) CRBM-CNRS UMR 5237, montpellier, FRANCE. (2) UMR 7091-LGN CNRS, villejuif, FRANCE.</p>
<p>To contact the author:: linda.cambier@crbm.cnr s.fr.</p>	<p>By quantitative hybridization of a high-density cDNA array, fourteen novel gene transcripts, called GENX, preferentially expressed in human muscles have been identified (Piétu et al., 1996). My research project is to identify and to characterize the cellular function of a protein, called PROTX-70612, encoded by one of these genes, localized on a human chromosome 6p21-31.</p> <p>Northern blot analysis has shown that this gene is specifically expressed in cardiac and skeletal muscles. Nevertheless, using RT-PCR, we show that it is in fact ubiquitously expressed in different adult mouse organs and that PROTX-70612 is ubiquitously expressed in these organs with a higher level in heart and brain.</p> <p>In the myogenic C2C12 mouse cell line, PROTX-70612 expression increases progressively during the differentiation process. Furthermore, down-regulation of PROTX-70612 expression in C2C12 cells using ShRNA and SiRNA techniques affects the expression of myosin suggesting that a disruption of PROTX-70612 expression inhibits muscle differentiation.</p> <p>Using indirect immunofluorescence microscopy and electronic transmission microscopy, we show that PROTX-70612 is localized in mitochondria of muscle cells. Mitochondria are organelles that play a central role in energy production for the cell and in the signaling of apoptosis. This localization can explain the high level of PROTX-70612 expression in organs like heart and brain, which consume a lot of energy. Furthermore, bioinformatic and biochemical analysis of the PROTX-70612 sequence show the presence of a N-terminal α-helix domain, which is necessary and sufficient to import the protein in mitochondria.</p> <p>Using submitochondrial fractionation techniques, we are defining the precise PROTX-70612 localization in the mitochondria. We are also trying to determine binding partners for PROTX-70612 that could indicate the signaling pathway in which the protein is implicated.</p> <p>Collectively, these results show the identification of a novel mitochondrial protein playing a role during the differentiation process of skeletal muscle cells.</p>

PW38-468	<p><u>EXPRESSION AND ROLES OF THE ORPHAN RECEPTOR ALK IN THE DEVELOPMENT OF THE NERVOUS SYSTEM</u> DEGOUTIN J¹, BRUNET-DE CARVALHO N¹, GOUZI JY², CIFUENTES-DIAZ C¹, VIGNY M¹ (1) Institut du Fer à Moulin UMR_S839 INSERM/UPMC, Paris, FRANCE. (2) BSRC 'Alexander Fleming', Vari, GREECE.</p>
<p>To contact the author:: degoutin@fer-a-moulin.inserm.fr.</p>	<p>Anaplastic lymphoma kinase (ALK) is a receptor tyrosine kinase that is transiently expressed in specific regions of the central and peripheral nervous systems, suggesting a role in its normal development and function. The nature of the cognate ligands of ALK in vertebrate is still a matter of debate. We produced a panel of monoclonal antibodies (mAbs) directed against the extracellular domain of the human receptor. Two mAbs induced the differentiation of PC12 cells transiently transfected with ALK. In HEK 293 cells stably expressing ALK, we showed that these two Mabs strongly activated the receptor and subsequently the MAP kinase pathway and a specific activation of STAT3. Interestingly, others mAbs present all the characteristics of blocking antibodies. We also studied the primary events occurring at the plasma membrane triggered by ALK activation and driving MAP-kinase activation and subsequently PC12 cells differentiation. We focused on two adaptor proteins Shc and FRS2, and their specific role in the neuron-like differentiation of PC12 cells. We showed that both adaptors could interact with ALK in an activation dependent manner. We also characterized the functional role of Shc adaptor in the neuron-like differentiation of PC12 cells. Recently, we studied the expression of ALK in DRG (dorsal root ganglia) neurons both in vivo and in culture. Our results on the level of expression showed a maximum reach at P0 (birth) in the rat DRG. Immunofluorescence assay on section of DRG showed a specific localization of ALK in a subtype of neurons. Moreover, our data suggested that ALK could be involved in the relation between neurons and Schwann cells.</p> <p>Thus, in absence of clearly established ligand(s) in vertebrates, the availability of mAbs allowing the activation or the inhibition of the receptor will be essential to better understand the roles of ALK.</p>

PW38-469	<p><u>PLASMINOGEN INDUCES AN INTRACELLULAR SIGNALLING ACTIVATION THROUGH ITS BINDING TO ALPHA-ENOLASE IN MYOGENIC CELLS.</u> DIAZ-RAMOS A¹, LLORENS A¹, LOPEZ-ALEMANY R¹ (1) Biomedical Research Institute of Bellvitge (IDIBELL), L'Hospitalet de Llobregat (Barcelona), SPAIN.</p>
To contact the author:: madiaz@idibell.org.	<p>The plasminogen activation (PA) system is a group of serine proteases that participate in tissue remodeling by degrading most of components of the extracellular matrix. Different studies have shown that PA system components (plasminogen and urokinase-plasminogen activator) play a role in myogenesis <i>in vitro</i> and in muscle regeneration <i>in vivo</i>. Alpha-enolase constitutes a receptor for plasminogen in several cell types, where it acts focalizing proteolytic activity on the cell surface. We have previously shown that alpha-enolase/plasminogen binding is required for myogenic differentiation, fusion, migration and invasion processes, in an <i>in vitro</i> model of myogenesis. Moreover, the blockage of alpha-enolase/plasminogen binding in an animal model of muscle regeneration impairs the regeneration process, indicating an important role of cell surface-associated plasminogen, in an alpha-enolase-dependent way.</p> <p>In the last years, evidences have appeared showing that plasmin(ogen) induces an intracellular response after binding to cell surface in several cell types, but it has never been evaluated in myogenic cells.</p> <p>We have analysed the intracellular signalling response of plasminogen binding in myoblasts C2C12 and in primary cultures of <i>Muscle Precursor Cells</i> (MPCs). Our results showed that plasmin(ogen) induces PI3K/AKT and MEK/ERK phosphorylation in C2C12 myoblasts and in MPCs. This activation is alpha-enolase/plasmin(ogen) binding-dependent because inhibitors of plasminogen-cell surface binding (as MAb11G1 and EACA) abrogates this activation. Plasminogen induced invasion and migration were also inhibited by MAb11G1 and EACA, suggesting an important role for alpha-enolase/plasminogen binding induced response in these processes.</p> <p>As alpha-enolase lacks a transmembrane or intracellular domain, it is presumed to act through association with other membrane proteins. Experiments are being performed to identify such molecular partner that could collaborate with alpha-enolase to transduce plasmin(ogen)-induced intracellular signalling.</p>

PW38-470	<p>A NEW MODEL FOR MICROTUBULE DYNAMIC INSTABILITY. DIMITROV A¹, QUESNOIT M², MOUTEL S³, CANTALOUBE I⁴, POÛS C⁴, PEREZ F¹ (1) CNRS UMR144 – Institut Curie, Paris, FRANCE. (2) Univ.Paris-Sud IFR141 - Faculté de Pharmacie / CNRS UMR144 – Institut Curie, Châtenay-Malabry / Paris, FRANCE. (3) Translational Research Department – Institut Curie, Paris, FRANCE. (4) Univ.Paris-Sud IFR141 - Faculté de Pharmacie, Châtenay-Malabry, FRANCE.</p>
To contact the author:: ariane.dimitrov@curie.fr.	<p>Microtubules are highly dynamic tubulin polymers essential for intracellular organization and cell division. They display a dynamic instability, alternating phases of growth and shrinkage separated by catastrophe and rescue transitions. Tubulin polymerises in a GTP-bound form and hydrolyses GTP in the polymer with a slight delay. This creates a GTP-cap at the growing end of microtubules that is essential to prevent microtubule catastrophes. Loss of the GTP-cap would promote catastrophe events while microtubule rescue would be due to yet uncharacterized stochastic events.</p> <p>The existence of a GTP-cap at the extremity of microtubules has however not yet been documented in vivo. We selected a recombinant antibody (MB11) that specifically recognizes GTP-bound tubulin conformation in microtubules and we show here that GTP-tubulin is indeed present at the plus end extremity of more than half of cellular microtubules. However we also observe an unexpected staining. This suggests a new model for microtubule dynamic instability. We anticipate that the conformational antibody MB11 will be important both to understand the regulation of microtubule dynamic instability and to find new proteins and drugs that modulate the conformation and the dynamics of the polymer.</p>

PW38-471	<p>OVERVIEW OF TOR1A-LINKED DYSTONIA IN FRANCE FRÉDÉRIC M¹, CLOT F², BLANCHARD A¹, DHAENENS CM³, LESCA G⁴, CIF L⁵, DÜRR A³, VIDAILHET M², SABLONNIERE B³, CALENDER A³, BRICE A², CLAUSTRES M¹, TUFFERY-GIRAUD S¹, COLLOD-BEROUD G¹ (1) INSERM, U827, Montpellier, FRANCE. (2) INSERM, UMR_S679, Paris, FRANCE. (3) INSERM, U837, Lille, FRANCE. (4) Hôpital Edouard Herriot, Lyon, FRANCE. (5) CHU Montpellier, Hôpital guy de Chauliac, Montpellier, FRANCE.</p>
To contact the author:: melissa.frederic@montp.i nserm.fr.	<p>Early-Onset Torsion Dystonia (EOTD) are rare movement disorders developing in childhood with a neurological origin. They begin in a limb and potentially spread to other parts of the body to become generalized. Molecular defect is known for only a subgroup, consisting of a unique and recurrent mutation (c.907delGAG) in the <i>TOR1A</i> gene. Although the molecular cause of this particular form of EOTD is well known, there is very little epidemiologic data regarding mutation carriers. We first investigated the incidence at birth of the mutation in a population of 12,000 newborns from South-Eastern France and found only one positive. Our results suggest that the prevalence of the disease in France is in the lowest estimations, compared to previous epidemiological surveys (about 1/10,000 to 1/30,000 among non-Jews). We then undertook the census of French <i>TOR1A</i>-mutation carriers and the assessment of clinically associated signs to realize an overview of this population. Collaborations were established between the French laboratories involved in the molecular diagnosis of <i>TOR1A</i>-linked dystonia (Lille, Lyon, Paris and Montpellier). Family history, clinical data and DNA samples were gathered for each of the 53 identified index cases and for their relatives. From these data, we estimate disease frequency to be at least 0.13:100,000 and mutation frequency of 0.17:100,000 in France. Haplotypes linked to the c.907delGAG <i>TOR1A</i> mutation were constructed based on the analysis of flanking microsatellites. The previously reported Ashkenazi-Jewish haplotype, known to be linked with a founder effect, was found in 11 families. Only three of the remaining unrelated families shared the same haplotype suggesting that the mutation probably occurred independently several times in the French population. Finally, it was the first exhaustive nation-wide study of a genetically ascertained population of <i>TOR1A</i> carriers. Our results confirm the scarcity of this disease in the French population.</p>

PW38-472	<p><u>GENETIC HETEROGENEITY OF EMERY-DREIFUSS MUSCULAR DYSTROPHY: RESPECTIVE CONTRIBUTION OF LMNA, EMD AND OTHER GENES.</u></p> <p>GUENEAU L¹, BEN YAOU R¹, DEMAY L³, LLENSE S², CHIKHAOUI K¹, TRABELSI M², BEUGNET C², DEBURGRAVE N², LETURCQ F², CHELLY J², RICHARD P³, BONNE G¹</p> <p>(1) Institut National de la Santé et de la Recherche Médicale, U582, IFR14, Institut de Myologie, PARIS, FRANCE. (2) AP-HP, Groupe hospitalier Cochin, Laboratoire de Génétique Moléculaire, Pavillon Cassini, PARIS, FRANCE. (3) AP-HP, Groupe Hospitalier Pitié-Salpêtrière, U.F. Myogénétique et Cardiogénétique, service de Biochimie Métabolique, PARIS, FRANCE.</p>
To contact the author:: l.gueneau@institut-myologie.org.	<p>Emery-Dreifuss Muscular Dystrophy (EDMD) is a rare autosomal or X-linked recessive condition, associating muscular dystrophy, joint contractures and cardiac disease. Mutations in 2 genes, <i>EMD</i> (emerin) and <i>LMNA</i> (Lamins A/C) encoding nuclear envelope proteins account for only 40% of EDMD cases, suggesting additional genetic heterogeneity. Our objective was to estimate the respective contribution of <i>LMNA</i> and <i>EMD</i> mutations among an EDMD cohort screened in our labs.</p> <p>We reviewed medical records of 2200 patients screened for <i>LMNA</i> and/or <i>EMD</i>. EDMD clinical criteria were the coexistence of muscle involvement in a humero-peroneal, proximal or diffuse distribution; early tendons contractures; conduction defect and arrhythmias with or without dilated cardiomyopathy. <i>EMD</i> and <i>LMNA</i> genes were analyzed by dHPLC/sequencing with a preliminary analysis of emerin protein (western-blot, immuno-histochemistry).</p> <p>656 patients matched with our EDMD inclusion criteria. This study revealed the respective proportion of <i>LMNA</i> mutations (176 patients, 27%), <i>EMD</i> mutations (48 patients, 7%) detected following an abnormal emerin expression. 90% of <i>EMD</i> mutations were truncating ones whereas 94% of those found in <i>LMNA</i> gene were non-truncating. No mutation of these 2 genes was identified for 432 EDMD patients (66%) underlining the large number of patients lacking genetic diagnosis. Of these, 28 patients (6.5%) were eventually found to be mutated in other genes, as EDMD clinically overlaps with several other myopathies (LGMDs, CMDs, collagenopathies...) thus highlighting the importance of an accurate and careful diagnosis of this disease. From 96 patients consistent with X-linked transmission and for whom emerin protein was normal, analysis of <i>EMD</i> gene did not identify any mutation, confirming that emerin protein analysis remains a powerful diagnostic tool to detect <i>EMD</i> mutations.</p> <p>We finally characterized a global cohort of 404 patients eligible to search new EDMD genes. Six informative families are currently being genotyped. Meanwhile, candidate genes are being sequenced among our global cohort.</p>

PW38-473	<p><u>CONTROL OF PLANAR DIVISIONS BY THE G-PROTEIN REGULATOR LGN MAINTAINS PROGENITORS IN THE CHICK NEUROEPITHELIUM</u> MORIN X¹, JAOUEN F¹, DURBEC P¹ (1) Institute of Developmental Biology of Marseille–Luminy, Centre National de la Recherche ScientifiqueUMR6216, Marseille, FRANCE.</p>
To contact the author:: morin@ibdml.univ-mrs.fr.	<p>The spatio-temporal regulation of symmetrical as opposed to asymmetric cell divisions directs the fate and location of cells in the developing CNS. In invertebrates, G-protein regulators control spindle orientation in asymmetric divisions, which generate progeny with different identities. We investigated the role of the G-protein regulator LGN (also called Gpsm2) in spindle orientation and cell-fate determination in the spinal cord neuroepithelium of the developing chick embryo. We show that LGN is located at the cell cortex and spindle poles of neural progenitors, and that it regulates spindle movements and orientation. LGN promotes planar divisions in the early spinal cord. Interfering with LGN function randomizes the plane of division. Notably, this does not affect cell fate, but frequently leads one daughter of proliferative symmetric divisions to exit the neuroepithelium prematurely and to proliferate aberrantly in the mantle zone. Hence, tight control of planar spindle orientation maintains neural progenitors in the neuroepithelium, and regulates the proper development of the nervous system</p> <p><i>The first two authors contributed equally to this work.</i></p>

PW38-474	<p>HETEROLOGOUS EXPRESSION AND FUNCTIONAL ANALYSIS OF HUMAN MYOTUBULARIN IN THE YEAST MODEL SYSTEM <i>S. CEREVISIAE</i> KANEVA G¹, LAPORTE J², WINSOR B¹, FRIANT S¹ (1) Unité Mixte de Recherche 7156 Centre National de la Recherche Scientifique – Université Louis Pasteur, Département de Génétique Moléculaire, Génomique et Microbiologie, Institut de Biologie Moléculaire et Cellulaire, Strasbourg, FRANCE. (2) Département de Neurobiologie et Génétique, Institut de Génétique, Biologie Moléculaire et Cellulaire, Illkirch, FRANCE.</p>
To contact the author:: galinaka@yahoo.fr.	<p>The human myotubularin gene (hMTM1) that is mutated in a severe congenital neuromuscular disease, X-linked myotubular myopathy (XLMTM), encodes a phosphatase specific for phosphatidylinositol 3-phosphate (PtdIns(3)P) and PtdIns(3,5)P₂. In <i>Saccharomyces cerevisiae</i>, Ymr1p is the hMTM1 orthologue and the unique member of the myotubularin family. In order to study myotubularin intracellular functions as well as the impact of disease-causing mutations, hMTM1 wild-type and mutants constructs were introduced in yeast. Heterologous expression of hMTM1 wild type led to an enlarged vacuole phenotype, while a phosphatase inactive mutant (C375S) had no effect. The results obtained suggest that dephosphorylation of PtdIns(3)P and PtdIns(3,5)P₂ by human MTM1 in yeast impairs membrane trafficking, in particular the trafficking of carboxypeptidase Y (CPY). Depending on the mutation carried there is a difference in the severity of the vacuole phenotype in both <i>wt</i> and <i>ymr1Δ</i> strains. Mutation N180K responsible for a very mild form of XLMTM provoked a phenotype similar to that of the wild type myotubularin, Mutation V49F responsible for severe XLMTM triggered an increase in vacuole size, although less pronounced, and mutation R421Q (severe XLMTM) did not have an effect when compared to the C375S phosphatase inactive mutant. Surprisingly, mutation R69C (intermediate form of XLMTM) caused an enlargement of the vacuole that was more pronounced than the wild-type construct, suggesting a possible dominant negative effect of the mutation in this system. Apart from establishing a suitable model for studying the function of human myotubularin, the present work sheds light on the possible causes of X-linked myotubular myopathy such as defects in membrane trafficking. Similar studies for other members of the myotubularin family such as hMTMR2, mutated in Charcot-Marie-Tooth peripheral neuropathy, could give information about the pathological processes related to other neuro-muscular diseases.</p>

PW38-476	<p><u>ACCURATE WORK-RATE MEASUREMENTS DURING IN VIVO MRS STUDIES OF HUMAN QUADRICEPS EXERCISING MUSCLE</u></p> <p>LAYEC G¹, BRINGARD A², VILMEN C¹, MICALLEF JP³, LEFUR Y¹, PERREY S², COZZONE P¹, BENDAHAN D¹</p> <p>(1) CRMBM CNRS 6612, Marseille, FRANCE. (2) EA 2991, Montpellier, FRANCE. (3) INSERM ADR 08, Montpellier, FRANCE.</p>
To contact the author:: gwenael.layec@univmed.fr.	<p>Reliable investigation of exercising muscle within a superconducting magnet requires the construction of dedicated ergometers in order to perform standardised exercise protocols and to record mechanical variables. A few ergometers designed for quadriceps dynamic exercise have been described, but the corresponding mechanical data typically rely on the constancy of imposed work rates which is not satisfactory. Given that we have reached a point in the field of muscle energetics where absolute measurements are warranted to take the area forward, we designed an ergometer, including two force and two displacement transducers, allowing dynamic and isometric knee extension within a Magnetic Resonance (MR) system and accurate measurements of power output.</p> <p>On the basis of repeated measurements, the force and displacement transducers accuracy was 0.5% for values ranging from 0 to 394N and 3 % for values ranging from 0 to 20 cm. In addition, measurements were not affected by magnetic field and we were able to distinguish mechanical output during eccentric and concentric phases of exercise. MRS experiments in exercising muscle were conducted in 8 subjects. They performed two standardized dynamic alternate leg extension exercises (25 and 35 % of MVC) while the corresponding metabolic changes were measured using ³¹P-MRS.</p> <p>The mean power output produced during both exercises were 62 ± 17 and 79 ± 12 W. The corresponding metabolic changes were significant with a 20 to 40% PCr depletion and an end of exercise pH ranging from 7.0 to 6.3 pH units.</p> <p>Overall, the present ergometer is MR compatible. Dynamic and isometric leg extensions are possible while power output can be accurately quantified separately during the concentric and eccentric phases of exercise. Such an ergometer should be useful for future metabolic studies conducted in control subjects and patients for whom muscle energetics is impaired. Therapy follow-up is also possible.</p>

PW38-477	<p><u>SAFETY AND EFFICACY OF REGIONAL INTRAVENOUS (RI) VERSUS INTRAMUSCULAR (IM) DELIVERY OF RAAV1 AND RAAV8 TO NONHUMAN PRIMATE SKELETAL MUSCLE</u></p> <p>TOROMANOFF A¹, CHEREL Y², GUILBAUD M¹, PENAUD-BUDLOO M¹, SNYDER R³, HASKINS M⁴, DESCHAMPS JY², GUIGAND L², PODEVIN G⁵, ARRUDA V⁶, HIGH K⁶, STEDMAN H⁷, ROLLING F¹, ANEGON I⁸, MOULLIER P¹, LE GUINER C¹</p> <p>(1) INSERM UMR 649, CHU de Nantes, Université de Nantes, Nantes, FRANCE. (2) INRA UMR 703, Ecole Nationale Vétérinaire, Nantes, FRANCE. (3) Department of Molecular Genetics and Microbiology department, University of Florida, Gainesville, USA. (4) School of Veterinary Medicine, University of Pennsylvania, Philadelphia, USA. (5) CHU Hôtel Dieu, Nantes, FRANCE. (6) Department of Pediatrics, University of Pennsylvania, The Children's Hospital of Philadelphia, Philadelphia, USA. (7) Department of Surgery, University of Pennsylvania, Philadelphia, USA. (8) INSERM UMR 643, CHU de Nantes, Institut de Transplantation et de Recherche en Transplantation, Université de Nantes, Nantes, FRANCE.</p>
To contact the author:: caroline.le-guiner@univ-nantes.fr.	<p>We developed in the nonhuman primate (NHP) a drug-free regional intravenous (RI) delivery protocol of recombinant adeno-associated virus (rAAV) 1 and 8 to an entire limb and compared it with the intramuscular (IM) delivery of the same dose of vector. We show that RI delivery of both serotypes was remarkably well tolerated with no adverse side effects. After IM, muscle transduction was restricted to the site of injection with a high number of vector copies per cell for rAAV1, whereas RI delivery resulted in lower vector copy per cell but detectable in the vast majority of muscles of the injected limb. The amounts of circulating infectious rAAV were similar for both serotypes and modes of delivery. At autopsy, up to 34 months post vector administration, similar biodistribution patterns were found for both vectors and modes of delivery, with numerous organs positive for vector sequence by PCR and Southern-blot. Altogether, we demonstrated that RI is a simple and efficient transduction protocol in NHP, resulting in higher expression of the transgene with a lower number of vector genomes per cell. However, regardless of the mode of delivery, concerns were raised by vector sequence detected at distant sites.</p>

PW38-478	<p><u>DROSOPHILA MODEL FOR STUDYING LEG MYOGENESIS: ROLE OF LADYBIRD, A HOMOLOG OF VERTEBRATE LBX1 GENE AND REGULATORY INPUTS OF FGF AND WG SIGNALING PATHWAYS</u></p> <p>MAQBOOL T¹, SOLER C², JAGLA T¹, JAGLA K¹</p> <p>(1) INSERM U384, Faculté de Médecine, Clermont- Ferrand, FRANCE. (2) School of Biosciences, Cardiff University, Cardiff, UNITED-KINGDOM.</p>
To contact the author:: Tariq.maqbool@u-clermont1.fr.	<p>Leg muscles of <i>Drosophila</i> display a unique vertebrate-like multi-fiber organization. They form a highly stereotyped pattern of dorsal and ventral multi-fiber muscle units, which are attached to the internal tendons in the adult leg (soler et al., 2004)</p> <p><i>Ladybird</i>, a homeodomain transcription factor, is found to be expressed specifically in the myoblasts that form leg muscles. It precedes that of founder cell marker <i>dumbfounded</i>. The <i>RNAi</i>-mediated attenuation of <i>ladybird</i> expression alters properties of developing myotubes affecting their inherent ability to grow and to interact with the internal tendons and epithelial attachment sites. It also leads to affected sarcomeric ultrastructure contributing to the reduced leg muscle performance and altered mobility of surviving flies. Furthermore, the over-expression of <i>ladybird</i> results in abnormal pattern of dorsally located leg muscles indicating different requirements for <i>ladybird</i> in dorsal versus ventral muscles. This differential effect is consistent with the higher level of <i>Ladybird</i> in ventrally located myoblasts and with positive <i>ladybird</i> regulation by extrinsic <i>Wingless</i> signaling from the ventral epithelium. Moreover, <i>ladybird</i> expression correlates with that of FGF receptor <i>Heartless</i> and the read-out of FGF signaling <i>DOF</i>. FGF signals set up the number of leg disc associated myoblasts and resulting fibers and when over-expressed are able to accelerate myogenic differentiation by activating <i>ladybird</i> prematurely and leads to ectopic muscle formation, implying that FGF signaling may also be cooperating with extrinsic <i>Wg</i> signaling to regulate <i>ladybird</i> in leg myoblasts (Maqbool et al., 2006).</p> <p>To determine transcriptional gene activity in leg myoblasts and tendon cells, a protocol was developed for dissection, dissociation of myoblasts and tendon cells. The investigation of the candidate genes from microarray analysis, and understanding of how the coordinated development of muscle and tendon is orchestrated at the gene transcriptional level is expected to shed light on the molecular basis of myo-tendonous dysfunctions observed in a large spectrum of myopathies.</p>

PW38-479	<p><u>REGULATION OF UTROPHIN A IRES-MEDIATED TRANSLATION BY GLUCOCORTICOID TREATMENT IN SKELETAL MUSCLE CELLS</u> MIURA P¹, CORIATI A¹, SARKAR M¹, ANDREWS M², HOLCIK M², JASMIN B¹ (1) Department of Cellular and Molecular Medicine and Centre for Neuromuscular Disease, Faculty of Medicine, University of Ottawa, Ottawa, CANADA. (2) Apoptosis Research Centre, Children's Hospital of Eastern Ontario, Ottawa, CANADA.</p>
To contact the author:: pmiur055@uottawa.ca.	<p>Glucocorticoids are currently the only drugs recognized to benefit Duchenne muscular dystrophy (DMD) patients. The mechanisms that underlie the beneficial effects still remain incompletely understood. In agreement with previous observations, we show here that treatment of myotubes with the glucocorticoid 6-alpha-methylprednisolone-21 sodium succinate (PDN) results in enhanced expression of utrophin A without concomitant increases in mRNA levels, thereby suggesting that translational regulation contributes to the increase. Previously, we established that the utrophin A 5'UTR contains an Internal Ribosome Entry Site (IRES) that is activated <i>in vivo</i> during muscle regeneration (J Biol Chem. 280:32997-3005, 2005). In the present study, using monocistronic and bicistronic reporter assays, we demonstrate that activity of this IRES is enhanced by PDN treatment. Analysis of polysomes from PDN treated cells show an increase in polysome association of endogenous utrophin A mRNAs and reporter mRNAs harbouring the utrophin A 5'UTR, while global translation rates were found to be depressed. Additional experiments identified a distinct region within the utrophin A 5'UTR that contains the inducible IRES activity and displays enhanced binding to multiple proteins following PDN treatment. Together, these studies demonstrate that a translational regulatory mechanism involving increased IRES activation mediates, at least partially, the enhanced expression of utrophin A in muscle cells treated with glucocorticoids. As part of this work we have also created several lines of transgenic mice harbouring the utrophin A IRES reporter in order to assess the tissue distribution of utrophin A IRES activity and its regulation in response to both PDN treatment and muscle regeneration <i>in vivo</i>. In addition, we are also investigating the involvement of the utrophin 3'UTR in regulating utrophin IRES activity. These studies may contribute to the development of drugs that specifically target the utrophin UTRs to drive increased expression of utrophin in the muscle fibers of DMD patients.</p>

PW38-480	<p><u>STRUCTURE-FONCTION ANALYSIS OF THE S.CEREVISIAE PROTEIN BCS1P WHICH HUMAN HOMOLOGUE IS INVOLVED IN SEVERAL MITOCHONDRIAL PATHOLOGIES</u></p> <p>NOUET C¹, TRUAN G¹, DUJARDIN G¹ (1) Centre de Génétique Moléculaire, Gif sur Yvette, FRANCE.</p>
To contact the author:: nouet@cgm.cnrs-gif.fr.	<p>In man, mutations in the gene <i>BCS1L</i> are responsible for pathologies with varying clinical presentations: GRACILE syndrome (iron overload in liver), Bjornstad syndrome (pili torti and sensorineural deafness) and several pathologies characterized by deficiencies in the bc1 complex. The human protein, Bcs1, is 40% identical to Bcs1p of <i>S.cerevisiae</i>. In yeast Bcs1p is located in the mitochondrial inner membrane with a large domain protruding into the matrix. Bcs1p is composed of an N-terminal domain required for import into mitochondria, a central domain of unknown function and a C-terminal domain characteristic of AAA proteins (ATPases Associated with various cellular activities). Bcs1p is required for the assembly of the Rieske/FeS protein within the complex <i>bc1</i>. However, the molecular mechanism by which Bcs1p mediates assembly remains unknown. My thesis project focuses on the use of yeast genetic tools to further our understanding of the function of the different domains of the protein. I have undertaken a structure-function analysis based on a strategy of random PCR mutagenesis. I obtained a collection of a hundred of point mutants displaying respiratory deficiency. The mutations identified by sequencing the <i>BCS1</i> gene affected all parts of the protein. Three mutants affecting sub-domains that are only conserved in the Bcs1-like proteins, have been further characterized biochemically. Moreover an UV mutagenesis led to the isolation of intragenic compensatory mutations. To explain the effects of the primary and compensatory yeast mutations and to further understand the organization and the interactions between the different domains of Bcs1p, we have produced an homology-based model of the yeast and human Bcs1 proteins.</p>

PW38-481	<p><u>COLLAGEN XV, A NOVEL FACTOR IN ZEBRAFISH NOTOCHORD DIFFERENTIATION AND MUSCLE DEVELOPMENT</u></p> <p>PAGNON-MINOT A¹, MALBOUYRES M¹, HAFTEK-TERREAU Z¹, H KIM R², SASAKI T³, THISSE C⁴, THISSE B⁴, INGHAM PW², RUGGIERO F¹, LE GUELLEC D¹</p> <p>(1) IBCP,UMR CNRS 5086, Université Lyon 1, IFR 128 Biosciences Gerland, Lyon, FRANCE. (2) MRC Centre for Developmental and Biomedical Genetics, University of Sheffield, Sheffield, UNITED-KINGDOM. (3) Max-Planck Institut für Biochemie, Martinsried, GERMANY. (4) IGBMC, CNRS/INSERM/Université Louis Pasteur, Illkirch, FRANCE.</p>
To contact the author:: aurelie.pagnon@univ-lyon1.fr.	<p>Muscle cells are surrounded by extracellular matrix, the components of which play an important role in signalling mechanisms involved in their development. In mice, loss of collagen XV, a component of basement membranes expressed primarily in skeletal muscles, results in a mild skeletal myopathy. We have determined the complete zebrafish collagen XV primary sequence and analysed its expression and function in embryogenesis. During the segmentation period, expression of the Col15a1 gene is mainly found in the notochord and its protein product is deposited exclusively in the peri-notochordal basement membrane. Morpholino mediated knock-down of Col15a1 causes defects in notochord differentiation and in fast and slow muscle formation as shown by persistence of axial mesodermal marker gene expression, disorganization of the peri-notochordal basement membrane and myofibrils, and a U-shape myotome. In addition, the number of medial fast-twitch muscle fibers was substantially increased, suggesting that the signalling by notochord derived Hh proteins is enhanced by loss of collagen XV. Consistent with this, there is a concomitant expansion of patched-1 expression in the myotome of morphant embryos. Together, these results indicate that collagen XV is required for notochord differentiation and muscle development in the zebrafish embryo and that it interplays with Shh signalling.</p>

PW38-482	<p><u>IN VITRO AND IN CELLULO EVIDENCES FOR ASSOCIATION OF THE SURVIVAL OF MOTOR NEURON COMPLEX WITH THE FRAGILE X MENTAL RETARDATION PROTEIN</u></p> <p>PIAZZON N¹, RAGE F², SCHLOTTER F¹, MOINE H³, BRANLANT C¹, MASSENET S¹ (1) Laboratoire de Maturation des ARN et Enzymologie Moléculaire, UMR 7567 CNRS-UHP Nancy I, VANDOEUVRE, FRANCE. (2) Institut de Génétique Moléculaire de Montpellier, UMR 5535, MONTPELLIER, FRANCE. (3) Institut de Génétique et de Biologie Moléculaire et Cellulaire, CNRS/INSERM/ULP, STRASBOURG, FRANCE.</p>
To contact the author:: nathalie.piazzon@maem.uhp-nancy.fr.	<p>The common neurodegenerative disease spinal muscular atrophy (SMA) is caused by reduced levels of the survival of motor neuron (SMN) protein. SMN associates with several proteins to form a large complex that is essential for the assembly and metabolism of spliceosomal U snRNPs. It is still not understood why reduced levels of the ubiquitously expressed SMN protein specifically cause motor neuron degeneration. Recently, several lines of evidence support additional neuron-specific functions of SMN in mRNA transport and translation regulation in neuronal processes. FMRP (<i>Fragile X Mental Retardation Protein</i>), the defective protein in Fragile X mental retardation syndrome, is thought to play a role in the transport of mRNPs from the nucleus to the cytoplasm and may be crucial in neurons to repress translation of specific mRNAs during their transport as silent mRNPs from the cell body to growth cones and synapses. Therefore, we examined possible relationships of SMN with FMRP. We observed granules containing both transiently expressed RFP-tagged SMN and GFP-tagged FMRP in cell bodies and processes of rat primary neurons of hypothalamus in culture. By immunoprecipitation experiment, we detected an association of FMRP with the SMN complex in human neuroblastoma SH-SY5Y cells and in murine motor neuron MN-1 cells. Then, by <i>in vitro</i> experiments, we demonstrated that the SMN protein is essential for this association. We showed that the C-terminal region of FMRP, as well as the conserved YG box and the region encoded by exon7 of SMN, are required for the interaction. Our findings suggest a link between the SMN complex and FMRP in neuronal cells.</p>

PW38-483	<p><u>ROLE OF SIX HOMEOPROTEINS IN THE ACQUISITION OF THE FAST GLYCOLYTIC PHENOTYPE DURING MOUSE EMBRYOGENESIS</u> RICHARD A¹, FAVIER M², GUILLET-DENIAU I², MAIRE P² (1) Université pierre et marie curie, Paris, FRANCE. (2) inserm U567, paris, FRANCE.</p>
To contact the author:: arichard@cochin.inserm.fr.	<p>Previous studies have shown the importance of Six homeoproteins in different steps of myogenesis. Six homeoproteins control Pax3, myogenin and MRF4 genes in the embryo as well as aldolase A gene which is expressed specifically in fast glycolytic fibres. Moreover Six1 accumulates preferentially in the nucleus of adult fast-glycolytic fibres and forced expression of Six1 and its Eya1 cofactor in adult soleus fibres was able to switch their phenotype toward a fast glycolytic one. To define the role of Six homeoproteins during the genesis of muscle fibre type diversity, we generated a double knock-out model. <i>six1</i>^{-/-} <i>six4</i>^{-/-} mice die at birth and show severe muscle hypoplasia. At the truncal level, musculature is drastically reduced and residual myofibres show a disorganized ultrastructure. To characterize the slow/fast phenotype of fibres formed in absence of Six homeoproteins, we have collected embryos from 14 days of development until birth, and we analyzed the expression of different myosin heavy chain isoforms using immunohistochemistry. In <i>six1</i>^{-/-} <i>six4</i>^{-/-} fetuses, the number of myosin slow positive fibres was greatly increased, showing an alteration of muscle patterning. Furthermore microarrays experiments showed that in absence of Six homeoproteins, the expression of a great number of genes involved in intramuscular calcium metabolism (e.g. calsequestrin 1,) as well as in the contractile apparatus of the fast-type fibres was down-regulated, whereas the expression of slow-type isoforms was not modified. In parallel, we performed primary cultures of satellite cells isolated from residual muscles. Myoblasts were able to proliferate quite normally, but fusion was dramatically impaired. As it was the case in residual muscles fibres, satellite cells showed a 20 to 100-fold decrease in genes expressed in fast-type fibres (MyhIIIX, MyhIIB...). Altogether our results demonstrate that Six1 and Six4 genes are necessary to switch on fast-type specific genes in myogenic cells during embryogenesis.</p>

PW38-484	<p><u>REPROGRAMMING RNA MESSENGER BY SPLICEOSOME MEDIATED RNA TRANS-SPLICING: EVALUATION IN THE CANINE MUCOPOLYSACCHARIDOSIS VII MODEL</u></p> <p>SACHOT S¹, ROLLING F¹, MOULLIER P¹, LE GUINER C¹ (1) Laboratoire de Thérapie Génique INSERM U649, Nantes, FRANCE.</p>
To contact the author:: sylvain.sachot@univ-nantes.fr.	<p>Background:</p> <p>An approach to restore protein function involves the recombination of two RNA molecules in <i>trans</i> by a mechanism named Spliceosome Mediated RNA <i>Trans</i>-splicing (SMaRT™), in which splicing occurs between two independently transcribed pre-mRNA molecules. SMaRT™ requires an RNA molecule, the Pre <i>Trans</i>-Splicing Molecule (PTM), introduced artificially in cells.</p> <p>An advantage of <i>trans</i>-splicing method is the small length of the PTM to transfer, allowing the use of viral vectors such as rAAV. This strategy would also preserve the natural regulation of genes and limit immune responses often seen after gene transfer in large animal models, in which high numbers of transgene copies are transferred per cell.</p> <p>Our current hypothesis is to determine if the reprogramming of defective genes can be done, firstly <i>in vitro</i>, by the delivery of 5', 3' and double PTM, which permit the replacement of an internal exon, in canine mucopolysaccharidosis VII (MPS VII) cells.</p> <p>Method:</p> <p>5', 3' and double PTM have been designed, associated with various binding domain (BD) from 25bp to 220bp, and tested for their ability to <i>trans</i>-splice with a target transcribed from an artificial GusB Minigene and with the physiologically expressed GusB pre-messenger RNA, in MPS VII cells.</p> <p>Results:</p> <p>Our data demonstrated that reprogramming of artificially and physiologically expressed GusB pre-messengers is feasible <i>in vitro</i> in the MPS VII canine model using 5' and 3' PTM, as seen by RT-PCR and Western Blot. We also show illegitimate reprogrammations in the GusB minigene context.</p> <p>Conclusion:</p> <p>We have identified several functional 5' and 3' PTM. Their ability to reprogram the physiologically expressed GusB messenger will allow us to generate double PTM, and test them for such a reprogramming. We are also planning to develop strategies to improve the <i>trans</i>-splicing reaction efficiency. Finally, the more active molecule will be tested in MPS VII dog after a scAAV8 intravenous injection.</p>

PW38-485	<p><u>COUPLING GLUTAMATE RELEASE TO VASODILATION IN VIVO</u> TIRET P¹, LECOQ J¹, CHAIGNEAU E¹, DUCROS M¹, KNOPFEL T², CHARPAK S¹ (1) U603 Laboratory of Neurophysiology and New Microscopies, Université Paris Descartes, Paris, FRANCE. (2) Laboratory for Neural Circuit Dynamics, Riken Brain Science Institute, Saitama, JAPAN.</p>
To contact the author:: pascale.tiret@univ-paris5.fr.	<p>Several techniques of human functional brain imaging measure changes in blood flow parameters to localize activated cerebral regions. At present, the study of the spatio-temporal coupling between neuronal activity and associated vascular parameters is becoming a hot topic. Analysis of this coupling requires to measure the dynamics of red blood cell (RBC) flow in individual capillaries that precisely irrigate activated neurons. In a recent study, Charpak's team has used two-photon laser scanning microscopy, <i>in vivo</i>, to measure blood flow parameters in control condition and during odor stimulation in the dorsal olfactory bulb of anaesthetized rat (Chaigneau et al., PNAS, 2003). They found that capillary vascular responses are odorant- and glomerulus-specific. My PhD. project has consisted in using two-photon microscopy, <i>in vivo</i>, to determine the involvement of astrocytes and of smooth muscle cells in triggering vascular response. Such a project has initially required to assess the validity of a recent hypothesis that proposes that astrocytes activation is sufficient to trigger vasodilation of arterioles. Using two experimental models, the rat and the G-CaMP2 transgenic mice, I have demonstrated that this hypothesis was wrong and that neuronal postsynaptic activation is a necessary step for neurovascular coupling (Chaigneau et al., Journal of Neuroscience, 2007). More recently, I have been investigating the cellular and molecular pathway(s) that couples : neuronal postsynaptic activation, astrocytes activation and vasodilation. Precisely, I am examining the intermediate role of NO in these pathways. This <i>in situ</i> analysis of the regulation of the vascular tone should bring new insights in the understanding of fMRI signals detected during neuronal activation in the normal and pathological brain.</p>

PW38-486	<p><u>DELAYED GDNF GENE DELIVERY IN THE STRIATUM OF 6-OHDA-TREATED RATS PROMOTES A REVERSIBLE INCREASE OF TYROSINE HYDROXYLASE BUT NOT DOPAMINE LEVELS.</u></p> <p>YANG X¹, MERTENS B², LEHTONEN E¹, VERCAMMEN L³, BOCKSTAEL O¹, CHTARTO A¹, LEVIVIER M⁴, BROTCHE J⁴, SARRE S², TENENBAUM L¹</p> <p>(1) Laboratory of Experimental Neurosurgery/IRIBHM ULB, Brussels, BELGIUM. (2) Department of Pharmaceutical Chemistry, Drug Analysis and Drug Information VUB, Brussels, BELGIUM. (3) Laboratory for Neurobiology and Gene Therapy, Katholieke Universiteit, Leuven, BELGIUM. (4) Laboratory of Experimental Neurosurgery ULB, Brussels, BELGIUM.</p>
To contact the author:: xyang@ulb.ac.be.	<p>A tetracycline (tet)-inducible adeno-associated viral vector expressing human GDNF cDNA (AAV-tetON-GDNF) was administered in the striatum of rats 5 weeks after lesioning by intrastriatal 6-hydroxydopamine injection. A significant tet-dependent improvement of motor symptoms was evidenced 4 weeks post-vector injection. At later time points, a partial behavioural recovery was observed in all groups but no further improvement could be evidenced in GDNF-treated animals. Fourteen weeks post-vector injection, tyrosine hydroxylase (TH) levels were significantly higher and striatal TH-positive reinnervation significantly increased in GDNF-treated rats but the striatal dopamine content remained unchanged. We show that the lack of functionality of the additional TH-positive fibers in GDNF-treated animals could be due to a defect in TH phosphorylation. In addition, the TH increase was not permanent since withdrawal of tet after 4 weeks, resulted in TH levels comparable to the control groups. These data suggest that delayed GDNF gene delivery stimulates some components of dopamine metabolism but does not rescue dopaminergic neurons.</p>

PW38-487	<p>IN SILICO IDENTIFICATION OF ABD-A TARGET GENES IN THE DROSOPHILA HEART FROM GENE EXPRESSION DATA</p> <p>ZEITOUNI B¹, TÉVY MF², AERTS S³, HERRMANN C¹, POTIER D¹, SÉMÉRIVA M¹, CAPOVILLA M², PERRIN L¹</p> <p>(1) Developmental Biology Institute of Marseille-Luminy (IBDML) CNRS UMR 6216, Marseille, FRANCE. (2) Dulbecco Telethon Institute, Dept. of Biology and Evolution, University of Ferrara, Ferrara, ITALY. (3) Laboratory of Neurogenetics, Dept. of Molecular and Developmental Genetics, University of Leuven, Leuven, BELGIUM.</p>
To contact the author:: zeitouni@ibdml.univ-mrs.fr.	<p>A number of inherited cardiomyopathies affect cardiac muscle organogenesis emphasizing the need to improve our knowledge of heart formation. Recent advances have shed light on patterning informations that lead to cardiac muscle formation. However, how the involved molecular mechanisms interact to regulate heart organogenesis remains to be elucidated.</p> <p>We have demonstrated that the homeotic (Hox) genes, encoding homeobox transcription factors are required for cardiac tube formation in Drosophila. The remarkable conservation, between vertebrates and invertebrates, of the mechanisms responsible for heart formation, warrants the choice of this model organism. To gain insight into the downstream genetic control responsible for cardiac cell differentiation, we have applied a whole-genome expression analysis of the cardiac tube, where the Hox gene abdominal-A (abd-A), differentially expressed according to the anterioposterior axis, is known to be required for working cardiac cell differentiation. By this procedure, we pointed out a large set of genes up-regulated in the functional part of the heart, and 15 genes were further validated by hybridation in situ to share the same heart expression pattern as that of Abd-A. This group of co-expressed genes is likely to be regulated by Abd-A and common transcription factors (TFs), our current approach aims at identifying putative direct AbdA cis-regulatory sequences from these heart target genes. The conserved non-coding sequences of these genes (from DNA alignments between the D.melanogaster and D.pseudoobscura genomes) were ranked according to a bioinformatic motif search pipeline based on conserved clusters of multiple Abd-A and known cardiac TF binding sites. By attaching the genomic sequence to a GFP reporter and expressing the construct in transgenic embryos, we show that 3 of 18 tested enhancers are new heart-specific enhancers. We are now determining whether they are direct Abd-A targets, demonstrating the role of a Hox gene in the realization of heart identity.</p>

